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Page 1 of 1

Docket No.: CYTH.002DV3

CUSTOMER NO. 20995

Applicant	:	Fraser, et al.
App. No.	:	10/614,644
Filed	:	July 7, 2003
For	:	SYSTEMS AND METHODS FOR TREATING PATIENTS WITH PROCESSED LIPOASPIRATE CELLS
Examiner	:	Leon B. Lankford, Jr.
Group Art Unit	:	1651

CERTIFICATE OF FAX TRANSMISSION

I hereby certify that this correspondence and all marked attachments are being transmitted via facsimile to the USPTO Central Fax No. (571) 273-8300 on the date shown below:

June 22, 2007

Eric S. Furman, Ph.D., Reg. No. 45,664

Transmitted herewith for filing and consideration in the above-referenced application are the following items:

(X) Interview Summary in four (4) pages.

(X) Six (6) references enclosed.

(X) Total pages in transmission: 50

The Commissioner is hereby authorized to charge any additional fees which may be required, now or in the future, or credit any overpayment to Account No. 11-1410.

Eric S. Furman, Ph.D.
Registration No. 45,664
Attorney of Record
Customer No. 20,995
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JUN 2 5 2007

CYTH.002DV3 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Fraser, et al.
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June 22, 2007
(Date)

Eric S. Furman, Reg. No. 45,664

INTERVIEW SUMMARY

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

The attached interview summary reflects the substance of the personal interview held between Examiner Lankford, the undersigned, Marc Hedrick, and Richa Nand on June 21, 2007.

SUMMARY OF INTERVIEW

Applicants wish to thank Examiner Lankford for the courtesy extended during the personal interview held on June 21, 2007 and the helpful comments made therein. During the interview between the undersigned, Marc Hedrick, Richa Nand and Examiner Lankford, the indefiniteness rejections under 35 U.S.C. § 112, second paragraph raised in the Office Action mailed January 29, 2007 were discussed. Based on the interview, it was agreed that a claim directed to: "a self contained adipose-derived stem cell processing unit, comprising a tissue collection container configured to receive unprocessed adipose tissue that is removed from a patient, wherein said tissue collection chamber is defined by a closed system; a first filter that is disposed within said tissue collection container, wherein said first filter is configured to retain a first component of said unprocessed adipose tissue and pass a second component of said unprocessed adipose tissue , such that said first filter separates said first component from said second component, and wherein said first component comprises a cell population that comprises adipose-derived stem cells and said second component comprises lipid, blood, mature adipocytes and saline; a cell collection chamber, which is configured to receive said first component comprising a cell population that comprises adipose-derived stem cells from said tissue collection container, wherein said cell collection container is within said closed system; a conduit configured to allow passage of said first component comprising a cell population comprising adipose-derived stem cells from said tissue collection chamber to said cell collection chamber while maintaining a closed system; a cell concentrator disposed within said cell collection chamber, which is configured to facilitate the concentration of said first component comprising a cell population that comprises adipose-derived stem cells, wherein said cell concentrator comprises a centrifuge or a spinning membrane filter; and an outlet configured to allow the aseptic removal of said concentrated population of cells that comprise adipose-derived stem cells, would likely overcome the rejections set forth in the Office Action dated January 29, 2007.

Applicants also discussed the need to make of record all office actions presented in related co-pending applications and six (6) references, which were submitted in related applications but which were not of record in the present application. In an effort to expedite prosecution of the application, the Examiner kindly agreed to review the office actions online and

copies of the six (6) references and refer to the same in a subsequent action, if Applicants provided a list of the pending applications and sent copies of the six (6) references by facsimile to the Examiner's attention.

Pursuant to the conversation at the interview, Applicants provide herewith Table 1, which lists the related co-pending applications and Applicants wish to draw to the Examiner's attention to the fact that U.S. Application No.'s: 10/242094 and 10/035,278 are docketed to different Examiners. The remainder of the co-pending applications are docketed to Examiner Lankford. As agreed at the interview, Applicants have not provided the Examiner with paper copies of the Office Actions in the co-pending applications listed below but are happy to do so should the Examiner require such.

TABLE 1

Application No.	Attorney Docket	Examiner	Title or Claims
11/584202	CYTH.002C1	Lankford	Self Contained Cell Processing Device/Methods of Use
10/783957	CYTH.002CP1	Lankford	Treatment of Cardiovascular Conditions
10/884861	CYTH.002CP10	Lankford	Treatment of Stroke and Related Disorders
10/884638	CYTH.002CP11	Lankford	Clinically Safe Adipose-Derived Regenerative Cells/Devices with Sensors
10/877822	CYTH.002CP2	Lankford	Device With Programmable Unit/Cell Culturing Chamber
10/871503	CYTH.002CP3	Lankford	Augmentation of Autologous Fat Transfer
10/885293	CYTH.002CP4	Lankford	Cell Carrier and Containment Devices Containing Adipose-Derived Stem Cells
10/884637	CYTH.002CP5	Lankford	Treatment of Musculoskeletal Disorders
10/884639	CYTH.002CP6	Lankford	Treatment of Renal Disorders and Diseases
10/885294	CYTH.002CP7	Lankford	Treatment of Inherited and Acquired Disorders of the Bone, Bone Marrow, Liver, and Other Tissues
10/884860	CYTH.002CP8	Lankford	Wound Healing
10/884871	CYTH.002CP9	Lankford	Treatment of Peripheral Vascular Disease and Related Disorders
10/614431	CYTH.002DV1	Lankford	Additive + Mixing Concentrated Populations of Cells with Adipose Tissue
10/614392	CYTH.002DV2	Lankford	Cooling Concentrated Cells Prior to Administration
10/614644	CYTH.002DV3	Lankford	Self-Contained Cell Processing Device with Filter and Cell Concentrator
10/614648	CYTH.002DV4	Lankford	Compositions of Unprocessed Adipose Tissue and Concentrated Adipose-Derived Stem Cells
10/614643	CYTH.002DV5	Lankford	Mixing Concentrated Adipose-Derived Stem Cells with Unprocessed Adipose Tissue
10/242094	CYTH.017A	Afremova	Preservation of Non-Embryonic Stem Cells from Non-Hematopoietic Tissues

			(Cryopreservation/Banking)
10/325728	CYTH.019A	Mohamed	Systems and Methods for Treating Patients With Collagen Rich Material Extracted from Adipose Tissue
11/317422	CYTH.2CP1CP	Lankford	Cell Loaded Prosthesis for Regenerative Intraluminal Applications
11/138083	CYTH.2CPCPCP	Lankford	Treatment of Cardiovascular Conditions

Further to the discussion at the interview, Applicants provide herewith copies of the following six references for consideration by the Examiner, which were submitted in related co-pending applications. Applicants thank the Examiner for consideration of these documents and reference to the same in any subsequent action.

TABLE 2

1. EP 0 418 979; Published March 27, 1991; Applicant Dr. Michele Zocchi
2. EP 0 448 770; Published October 2, 1991; Applicant Katsuya Takasu
3. EP 0 515 726; Published December 2, 1992; Applicant Katsuya Takasu
4. US 4,834,703; Date of Patent May 30, 1989; Dubrul, et al.
5. Fulton, et al. "Fat Grafting." Fundamentals of Cosmetic Surgery. Fulton Skin Institute, Tustin, California. 19(3): 523-530 (July 2001).
6. Nguyen, et al. "Comparative Study of Survival of Autologous Adipose Tissue Taken and Transplanted by Different Techniques." Study of Transplanted Adipose Tissue. Plastic and Reconstructive Surgery. 85(3): 378-386 (March 1999).

No fees are believed to be due at this time, however, should a fee be due, please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Jun. 22, 2007

By: ______________________
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(54) Method and apparatus for producing human autologous collagen.

(57) This invention provides a method and the apparatus for producing human autologous collagen from the human fat tissues and specifically for a method of disintegrating fat cells using ultrasonic energy and separating fats, blood, water and serum from the collagen, all of which can be accomplished without removing material from the harvesting syringe. The purpose of the invention is to produce autologous collagen which is useful in several cosmetic procedures used to treat all the problems caused by ageing. In accordance with the procedure taught in the invention after fat cells have been removed from the subject in a harvesting syringe, first the material in the syringe is subjected to an ultrasonic energy source of a range between 20 kHz and 3mHz which destroys the fat cells by rupturing them which allows the oil in the fat cells to be separated from the residual cell walls, after which the harvesting syringe is placed in a specially designed centrifuge allowing for the blood, serum, fat cell oil and collagen to be completely separated. In the fat cell disintegration step, when applying ultrasonic energy, two alternative devices may be employed with the harvesting syringe containing the material. In one, the syringes are placed in a container filled with a cold liquid and subjected to ultrasonic energy. The second, preferred method utilizes a high intensity ultrasonic processor that includes a probe that can be placed in the harvesting syringe that is mounted individually in a metal support filled with a cold liquid. In the separation stage, a multi-bodied centrifuge is provided which holds up to four harvesting syringes safely while subjected to centrifugal action to permit the separation of the materials as described herein. With the use of the present invention human autologous collagen can be produced in minutes without removal from the harvesting syringe for use in the operation at the time or for frozen storage in an individual patient collagen bank.

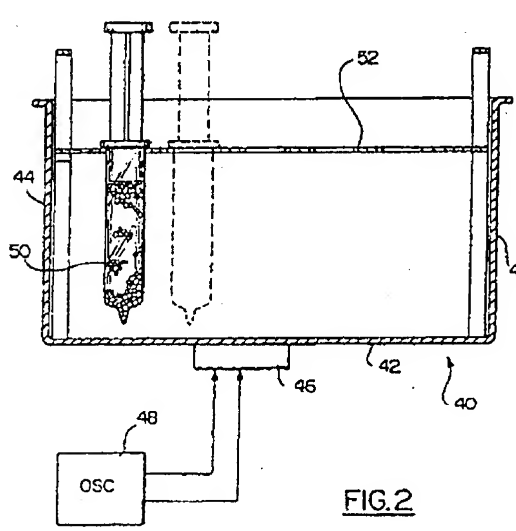

FIG.2

EP 0 418 979 A2

METHOD AND APPARATUS FOR PRODUCING HUMAN AUTOLOGOUS COLLAGEN

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method and apparatus for producing human autologous collagen from the human fat tissues, and specifically to an improved method and apparatus for producing collagen without removing the treated tissues from the harvesting syringes used for the harvesting allowing in this way to complete the whole procedure in a sealed sterile cycle.

2. Description of the Prior Art

Fat transplantation, better known as lipofilling is known to be one of the most efficient cosmetic procedures to treat the problems caused by ageing. The surgical process involves the harvesting of autologous fat tissue and the transfer of the harvested fat tissue to the facial or body areas requiring correction after the tissue is separated from water, saline, blood serum and physiological and aesthetic solutions injected before the surgery. In using this surgical procedure, one of the choices (implant or graft) is to inject just the autologous collagen contained in the cell wall of adipocytes. The problem in using this approach has been in obtaining the collagen necessary to complete the surgical process. In this case, it is an implant and not a graft, and thus the permanence of the material in the injected areas is assured not by a cellular survival but by its progressive integration in the conjunctival structures of the implantation zone.. It is just for the experienced use of this last technique, sometimes combined with the first one, that the author has created a new method assuring the achievement of a dense and homogenous product and of the excellent quality, with a complete utilization of the harvested fat tissue. Collagen is an insoluble fibrous protein that occurs in vertebrates as the chief constituent of the fibrils of connective tissue such as skin. To date one process for obtaining collagen was very time consuming and inefficient which involved mixing harvested tissues with distilled water which by its hypotonic capacity increased the volume of the fat cells until they were partially ruptured and destroyed. Another alternative has been to use bovine collagen, a commercial product obtained by treatment of cow's skin. This is a very expensive procedure that cannot be used with all the patients due to the severe allergical reactions to this product.

The present invention overcomes the problem of producing autologous collagen without removing it from the harvesting syringes by the application of ultrasonic energy which acts to destroy the fat cells allowing the mixture to be separated by centrifuge with a highly dense purified collagen resulting from the centrifugal action on the mixture. The invention also embodies an improved centrifuge for use in the separation process.

SUMMARY OF THE INVENTION

A process and system for producing autologous collagen comprising the harvesting of fat tissue from the patient with the use of aspirating syringes, subjecting the syringes containing the harvested material to ultrasonic energy until the thorough and complete destruction of the fat cells is accomplished, and separating the resultant material in the syringe in a centrifuge which allows the collagen to be separated out from the remaining mixture for resultant injection. In detail it is possible distinguish in the syringes, from below to high, three different layers;

1) in the lower part the blood, the physiological saline and anaesthetic solution used for the harvesting and the rinsing;
2) in the middle, the auto-collagen formed by all cellular residues, the wall of the burst adipocytes and intercellular substances, very isolated;
3) on the surface, a very important oily layer coming from the lipidic matrix of the burst cells.

It is clear that only the middle layer, formed by the autologous collagen interests us for the reinjection. It is very simple by a needle to remove all parasitic layers and so to obtain in all syringes a pure collagen substance.

It is also interesting to note that the process of centrifugation using the centrifuge invented by applicant and described herein can also be used in the traditional lipofilling (fat tissue transplant). The centrifugal force does not cause any structural change on the adypocites and does not affect their capacity of surviving. On the contrary, it considerably reduces times of their manipulation and rinsing, thereby making it possible to regraft them very quickly in the areas to be improved, such that the cells to begin again immediately vascular and metabolic exchanges.

In one embodiment of the method of the invention, with respect to the application of ultrasonic

energy to be harvested fat tissue, the syringe containing the harvested fat tissue receives a sterilized ultrasonic probe which exposes the harvested material in the syringe to an appropriate ultrasonic frequency such as approximately 20 kHz for a period of time up to 20 to 25 seconds. The source of ultrasound waves changes the electrical current from 110/220 V to 50/60 Hz in an electric energy with a highest frequency from 20 kHz to 3 mHz (20,000-3,000,000 cycles/sec.). This energy at high frequency is transmitted later to the piezo-electrics transducers where it is transformed in mechanical vibrations.

These vibrations are intensified by a resonance chamber by synergy within different transducers (1 or more) and then transmitted to the contained liquid, so creating some waves of pressure.

This operation causes the formation of millions of micro-blisters which increase during the pressure drop wave and implosent violenty during the positive pressure wave. It is exactly this physical phenomenon, better known with the technical term of cavitation, which produces this great action of cellular fragmentation. During cavitation free radicals are formed which, if they are allowed to accumulate, can greatly affect the biological integrity of the sample. Although during short periods of processing their formation is not normally considered a problem. To avoid this side effect the probe is immersed in the the harvested tissues contained by the syringe through the top of the syringe after removing the piston and held in a vertical position in a stainless steel container filled with cold sterile saline mixed with alcohol to allow lower temperatures.

Another alternative is to saturate the solution with hydrogen or carbon dioxide that often eliminates free radical formation. In this case a small pellet of dry ice dropped in the solution will often resolve the problem. It is important to avoid the overheating of the tissues (not to exceed 60 C°) due to the energy provided by the ultrasonic probe.

During such a process, the coolant solution in which the syringes are suspended absorbs any excess heat generated by the process, avoiding the possibility of overheating the material processed which might cause unwanted alteration of such material. After the period of exposure to the ultrasonic energy, the tissues come out finely fragmented and homogenized. After the probe has been removed, the syringe is transferred to a centrifugation process which separates the collagen from all the other harvested materials. It is applicant's position that exposure to the proper ultrasonic frequency for the correct amount of time acts to disintegrate the cell walls by implosion allowing the oil in the cells to escape. Because of the action of a centrifuge, the different densities of the harvested materials and ruptured cells permit a clear and defined separation in different layers of collagen and the remaining materials.

In an alternate embodiment, a plurality of syringes containing harvested material may be put into a container filled with a cold sterile saline solution. The walls of the container transmit the action of several piezo electric transducers generating vibrations at a frequency between 20 kHz and 3 Mhz for a period of time of between 15 and 25 minutes. Again, once the ultrasonic vibration energy has destroyed the fat cells completely, the syringes are placed in a centrifuge for separation. The cold saline solution prevents the harvested material from becoming overheated.

When utilizing a probe for ultrasonic energy that is immersed into the harvested material contained by the syringe for the total disintegration of the fat cells, the probe may be based on ultrasonic processors that are currently for sale that have been used previously for emulsification and the like of laboratory samples. One such example is a product known as Vibra Cell that is sold by Sonics and Materials. Inc., Danbury, Connecticut. One other example is a product known as Lipotrit 1 that is designed by the inventor and sold by Bielsan S.r.l. Electro-Medical Equipment, Milan, Italy.

With respect to the centrifuge, applicant's invention also includes an improved centrifuge in which four separate chambers are provided such that each chamber houses a syringe without the needle but with the piston in full extension to ensure the proper spinning action without damage to the syringe itself.

It is an object of this invention to provide an improved method for producing autologous collagen for use in the implantation during liposculpturing surgery of the human body.

It is an object of this invention to provide apparatus for the production of collagen for use in the implantation in liposculpturing surgical procedures of the human body.

It is yet another object of this invention to provide an improved process and equipment that greatly reduces the cost and time required to collect autologous collagen from human fat cells which are used for aesthetic surgery procedures on human beings.

Yet still another object of the invention is to provide a method and apparatus for producing human collagen which is obtained in a closed and sterile environment rapidly and with simplicity by the surgeon himself while performing a lipofilling procedure or which may be frozen and preserved in a sealed and sterile envelope to develop a collagen bank for the patient.

In accordance with these and other objects which will be apparent hereinafter, the instant in-

vention will now be described with particular reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a, 1b, 1c show a schematic diagram of a syringe which houses the harvested material throughout the disintegration and separation stages of the process.
Figure 1d shows a schematic diagram of the apparatus used to ultrasonically disintegrate the fat cells prior to separation.
Figure 2 shows an alternate embodiment in a schematic diagram for providing the ultrasonic disintegration of fat cells prior to separation.
Figure 3 shows a side elevational view of a centrifuge used with the present invention.
Figure 4 shows a top plan view of the centrifuge shown in Figure 3.

PREFERRED EMBODIMENT OF THE INVENTION

The method in accordance with the preferred embodiment of the invention comprises the steps of harvesting human fat tissue (which includes autologous collagen) from the patient, collecting the fat cells in a syringe, surrounding the syringe with a coolant, subjecting the harvested material while it remains in the sterile environment of the syringe to an ultrasonic energy force that acts to disintegrate and destroy the fat cells by rupturing the cell walls within the syringe, and subjecting the syringe with the material to centrifugation which allows for separation of the harvested tissue into layers being such that the collagen will collect in a predetermined layer.

The preferred apparatus for applying ultrasonic energy to the harvested material in the syringe, is shown in Figure 1d. Figures 1a, 1b and 1c show a typical syringe 60 having harvested fat cells 62. Figure 1a shows a conventional syringe 60 used to collect or harvest human fat cells that includes a plunger 60a and needle 60b. Figure 1b shows the syringe 60b with harvested fat cells 62 received from a patient. In order to subject the harvested cells 62 with the ultrasonic energy, the plunger 60a and needle are removed from syringe 60 as shown in Figure 1c. The opening that provides for the needle 60b attachment is covered and sealed by plug 70. In Figure 1d, the syring 60 is placed in a stainless steel support vessel 72 containing cold sterilized liquid (saline and alcohol solution) that surrounds a large portion of the syringe body 60. The cold liquid ensures that the harvested material 62 will not become overheated because of the application of ultrasonic energy transmitted from probe 68 extending from upper body portion 66. The probe 68 extends from ultrasonic wave generator that produces energy at 20 kHz in the harvested material 62 for 2 to 3 minutes for volumes of 10cc to 60 cc or as required. Such an ultrasonic device could be the vibra cell VC50 which is sold by Sonics and Matèrials Inc. of Danbury, Connecticut or the LIPOTRIT 1 designed by the inventor and sold by Bielsan, S.R.1. Electro-Medical Equipment, Milano, Italy. As shown in Figure 1d, such ultrasonic energy device 66 includes a pre-sterilized probe 68 which is disposed in syringe 60 containing harvested cell material 62 with the probe 68 being disposed through the top of the syringe 60. In the preferred embodiment the material 62 in the syringe 60 will be exposed for a period of time of approximately twenty to thirty seconds to the actionof the probe 68 which produces vibrations of a frequency of approximately 20 kHz in the harvested material causing cavitations of high and low pressure wave energy which acts to disintegrate and rupture the fat cell tissues in a very short period of time. Once the total disintegration of the fatty tissue is complete, the probe 68 is removed from the syringe and the syringe then placed in a centrifuge as is discussed below.

Figure 2 shows an alternate embodiment of the invention which also provides ultrasonic energy to a syringe containing harvested material. In Figure 2 the syringe 50 is shown mounted in a rack 52 all of which is mounted in an ultrasonic transmitting container 44 which is attached to a transducer 46 coupled to a source of oscillating energy 48 along the bottom 42 of container 44. The container may also include a suitable cold sterile solution (saline and 60% alcohol) to prevent the harvested material from becoming overheated from the ultrasonic energy (not to exceed 60° c). The harvested material in syringe 50 (the container 44 could hold several syringes) is subjected then to an ultrasonic energy from 20 kHz to 3 mHz for a period of time of approximately fifteen to twenty minutes. Again the fat tissue is disintegrated by the cavitation action created by high and low sonic pressure waves within the material causing cells to rupture and be destroyed. After the process of treating the harvested materials in the syringes with ultrasonic energy is complete, the syringes are then removed to a centrifuge that is described below. The plunger or piston 60a is replaced in each syringe prior to centrifugation.

Referring now to Figures 3 and 4, the centrifuge in accordance with the present invention is comprised of base 10 which supports a rotatable vertical shaft 12 driven by an electric motor with conic transmission gears 16 and 18. The electric motor 14 is connected to a timer 20 which can

supply electrical energy to the motor 14 for a desired length of time and which can be adjusted in time by button 22. The upper end of shaft 12 extends above the base 10 and has a support platform 24 which is rotatable and made preferably of stainless steel or other comparable material that can be sterilized at high temperatures. The support platform 24 includes a square member 26 and four extending syringe receiving chambers 28, 30, 32 and 34 branching outwardly, each chamber of which is formed with an aperture facing toward the axle 26a of the square member 26 and each chamber is spaced in relation to the aperture of the opposing chamber. The chambers 30 and 34 are positioned higher than chambers 28 and 32 relative to the axle 26a of the centrifuge. In the preferred embodiment the chambers are slightly tilted in relation to the horizontal position for about fifteen degrees to prevent the syringes inside from falling out. The four chambers 28, 30, 32 and 34 receive sterile test tubes 36 which houses each of the respective syringes. By staggering the openings of the syringe receiving chambers vertically, four loaded syringes, with pistons in full extension, can be inserted simultaneously. In the beginning of the process the shaft speed of the centrifuge is about 1000 R.p.m. and can be between 500 and 2000 R.p.m. for the longest radius of the chambers approximately 15 centimeters between the axle, the device and the bottom of the chamber. The action of the centrifuge can provide a perfectly stratified and completely pure mixture of collagen after a centrifugation of less than 20 seconds, which is a density and purity not realized by any previous procedures. After the centrifugation action, the syringes are then removed and the non-collagen materials are removed by use of another sterilized syringe needle. The remaining material is a highly purified collagen ready for injection or frozen storage in a patient collagen bank.

Because of applicant's invention described herein, implantation of autologous collagen for cosmetic surgery now becomes a practical procedure resultant from the availability of low cost quantities of autogolous collagen produced by applicant's invention.

The instant invention has been shown and described herein in what it is considered to be the most practical and preferred embodiment. It is recognized, however, that departures may be made therefrom within the scope of the invention and that obvious modifications will occur to a person skilled in the art.

Claims

1. A method for producing collagen comprising the steps of:
collecting a sample of harvested fat tissue in a syringe;
subjecting the harvest material contained within the syringe to ultrasonic energy for a prescribed time period to destroy the fat cells therein; and
separating the harvested material after it has been subjected to ultrasonic energy with centrifugal force.

2. The method as in claim 1, wherein the ultrasonic energy is between 20 kHz and 3 (mHz).

3. The method as in claim 1, wherein:
the syringe containing the harvested fat tissue has ultrasonic energy transmitted therein to the harvested material through a probe immersed in the syringe without the piston.

4. The method as in claim 1, wherein:
ultrasonic energy is applied to the syringe by placing the syringe in a container having one or more ultrasonic transducer attached thereto; said container including a liquid for removal of heat around the syringe.

5. A device for producing collagen from harvested fat tissue comprising a syringe containing harvested fat tissue;
an ultrasonic probe which is receivable into said syringe for destroying fat tissue contained in said syringe;
power supply means for providing power to said ultrasonic means; and
centrifuge means for receiving said syringe containing said harvested material for separating collagen from said remaining destroyed fat tissues.

6. A device as in claim 5, wherein:
said centrifuge includes a plurality of separately spaced chambers mounted with chamber openings having the longitudinal axes of the chambers being set rectangularly spaced equally around the central axis of a mounting plate;
means attached to the mounting plate for rotating the chambers which receive syringes;
power supply means for rotating said mounting plate connected to said mounting plate whereby syringes containing harvested fat cells having been subjected to ultrasonic energy are subjected to centrifugal force providing separation of said human oil, and other impurities from the collagen whereby the collagen will be collected in a well separated layer.

7. A centrifuge for use in producing collagen from harvested human fat tissues or to condense and stratify the intact fat cells for grafting, comprising:
a rotatable rectangular platform
means connected to said platform for rotating said platform;
a plurality of elongated hollow tubes having a closed end and an open end, said tubes being sized to receive a syringe, said hollow end being

rigidly fixed adjacent one edge of said rectangular rotatable platform such that reciprocal tubes have hollow openings facing each other but said reciprocal tubes are off center aligned so that each of said tubes can be filled with a syringe simultaneously.

8. A centrifuge as in claim 7 wherein:
said rigid platform is substantially mounted horizontally parallel to the earth and said tubes are inclined at a slight angle relative to the horizontal plane downwardly from their opening.

9. A method for producing collagen comprising the steps of:
collecting a sample of harvested fat tissue;
subjecting the harvested material to ultrasonic energy; and
separating the harvested material after it has been subjected to ultrasonic energy.

10. The method as in claim 8, wherein:
the separation is done by centrifugal force.

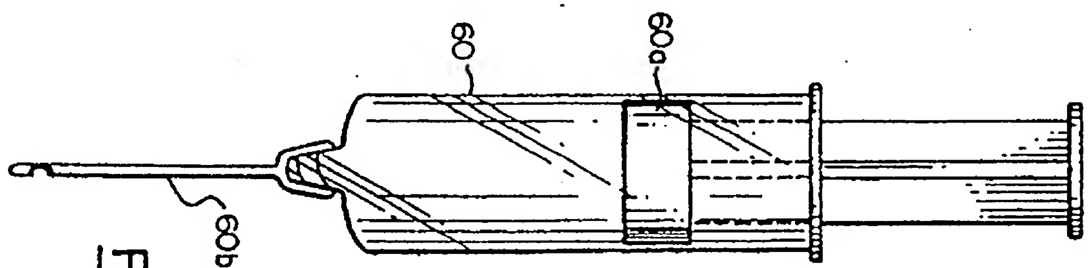

FIG. 1A

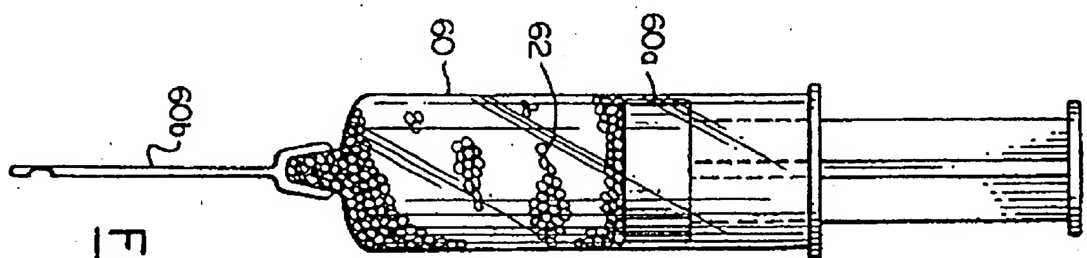

FIG. 1B

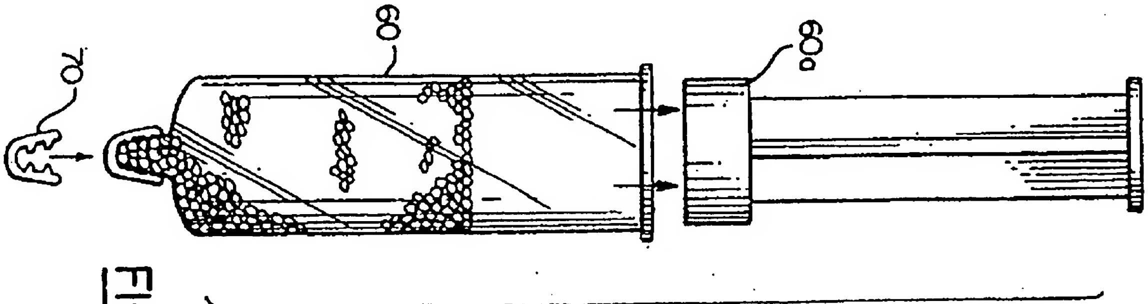

FIG. 1C

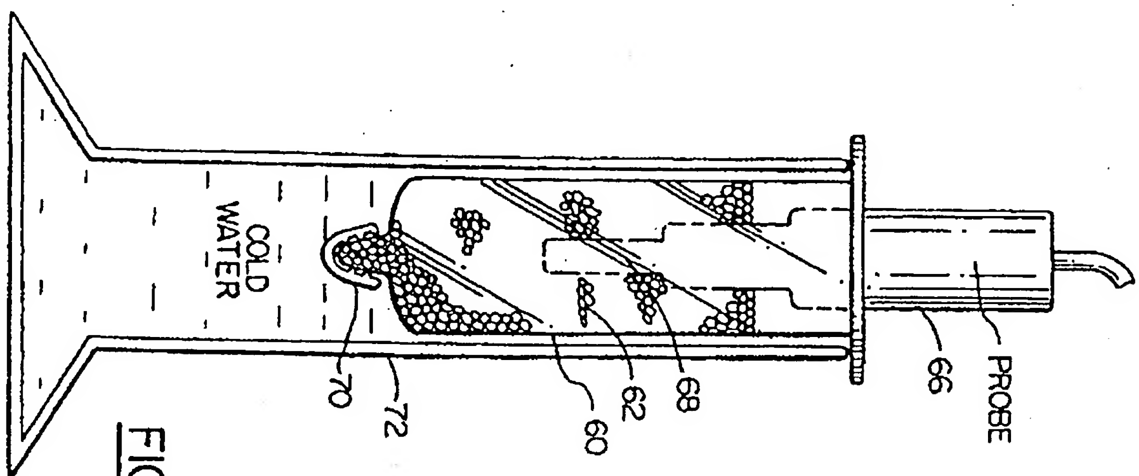

FIG. 1D

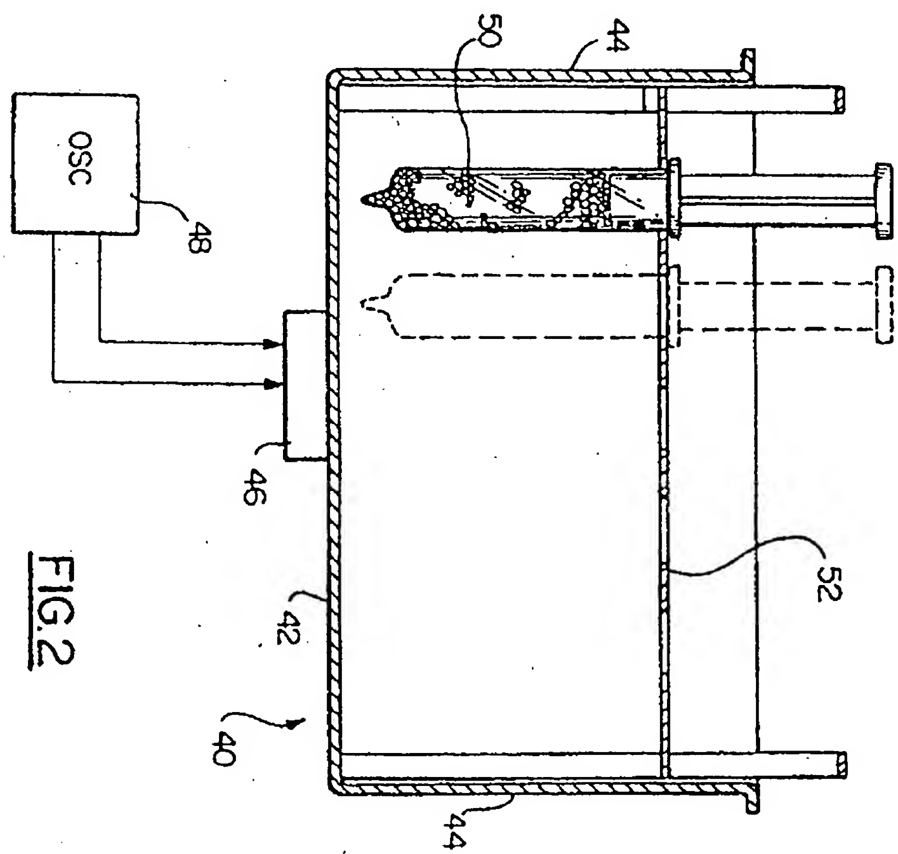

FIG. 2

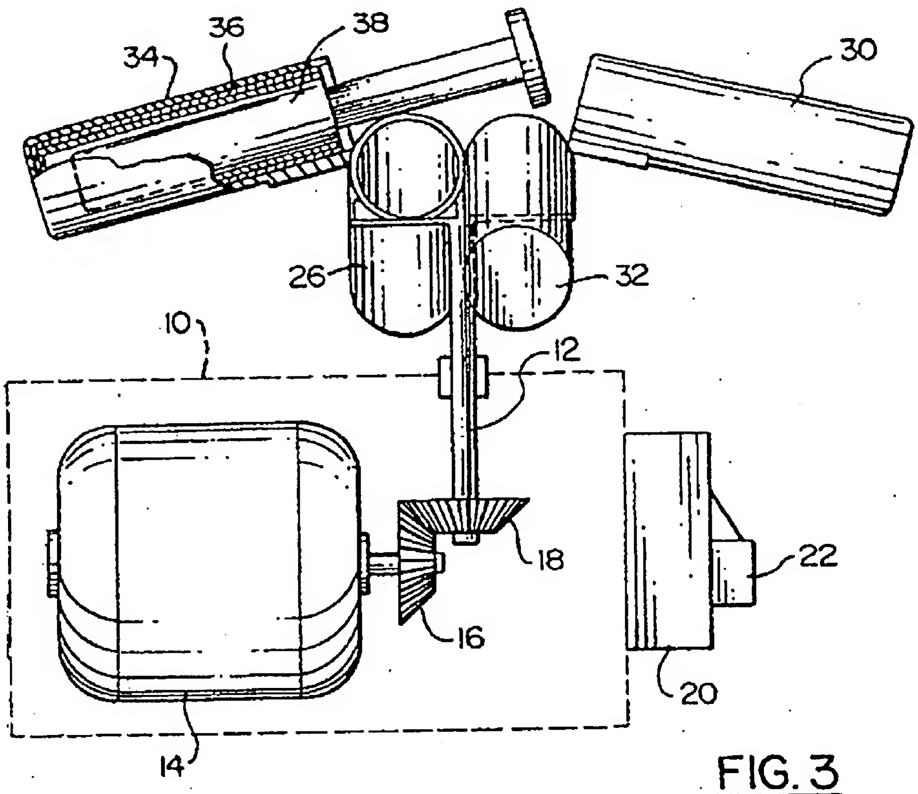

FIG. 3

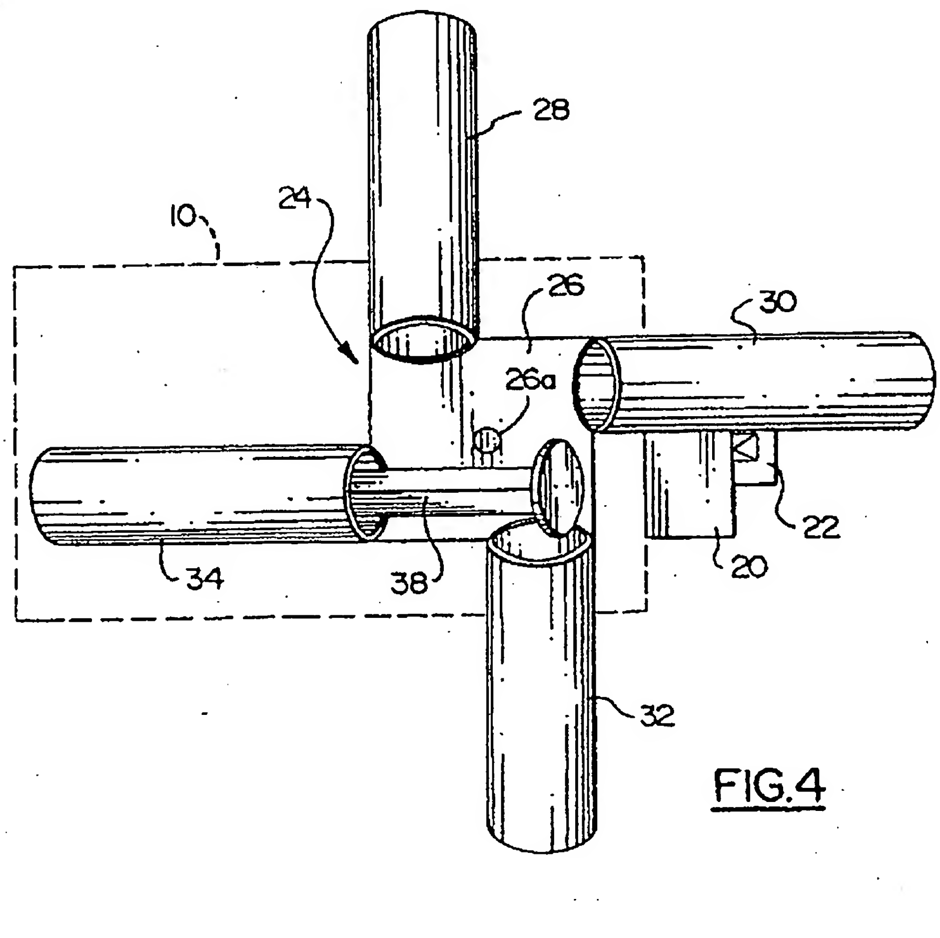

FIG.4

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(54) Method for separating collagen.

(57) A method for separating collagen including adding distilled water to a piece of fat which is extracted from a human body, so that the fat cells are destroyed by the osmotic pressure of the distilled water, and separating collagen from a fat component which has a smaller specific gravity than that of the collagen.

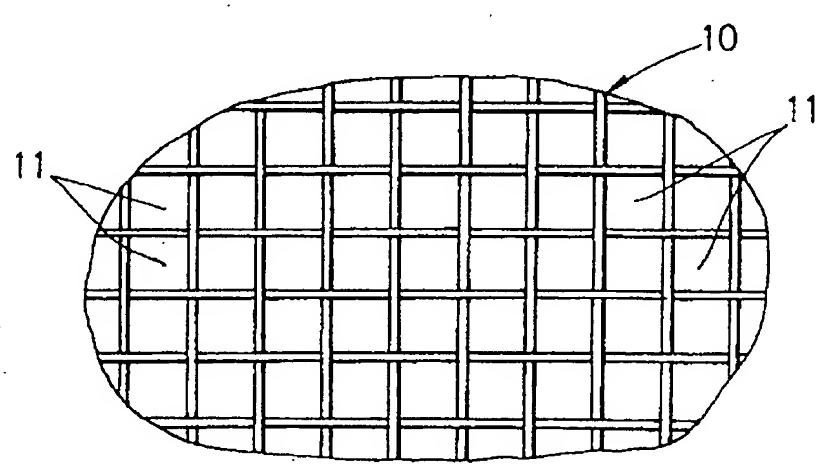

FIG-1

EP 0 448 770 A1

The present invention relates to a method for separating collagen, and more precisely, it relates to a method for separating collagen from fat of a human body.

In cosmetic surgery, it is known to inject collagen into a patient's skin to remove or remedy senile lines (wrinkles) or depressions, such as a scar. Collagen which is a main component of a connective tissue which constitutes bones and skin, etc. is made of fibrous protein. Accordingly, the skin with depressions or the wrinkled skin can be flattened or remedied by the collagen which is directly injected into the skin with an injector.

Collagen which is used in such conventional cosmetic surgery is usually extracted and refined from cowhide. However, since the collagen is a heterogeneous protein from a human body, there is a possibility of an allergic reaction.

The inventor of the present invention has focused on a technology of fat extraction from a human body which is widely used in a cosmetic surgery. Namely, the inventor has conceived a separation of collagen from the extracted fat. In case of collagen which is separated from his or her own fat, no allergic reaction occurs , unlike the prior art in which an allergic reaction occurs owing to a hetelogeneous protein, as mentioned above.

The primary object of the present invention is to provide a method for effectively and simply separating collagen from fat of a human body.

To achieve the object mentioned above, four different collagen separation methods are provided according to the present invention. These are a first separation method by distilled water, a second separation method by freezing, a third separation method by filtering, and a fourth separation method by ultrasonic oscillation.

According to a first aspect (first separation method) of the present invention, there is provided a method for separating collagen comprising adding distilled water to a piece of fat which is extracted from a human body, so that the fat cell is destroyed by the osmotic pressure of the distilled water, and separating collagen from a fat component which has a smaller specific gravity than that of the collagen.

Preferably, the separation of the collagen from the fat component is effected by a centrifugal separation.

According to a second aspect (second separation method) of the present invention, there is provided a method for separating collagen comprising adding distilled water to a piece of fat which is extracted from a human body, quickly freezing the mixture of the fat piece and the distilled water to a temperature below -50 °C to destroy the fat cells, and then separating collagen from a fat component which has a smaller specific gravity than that of the collagen.

According to a third aspect (third separation method) of the present invention, there is provided a method for separating collagen comprising adding a small amount of distilled water to a piece of fat which is extracted from a human body, and filtering the mixture of the fat piece and the distilled water to separate collagen from the fat cells.

Preferably, the separation of the collagen from the fat cells is effected by a filter which has a large number of openings of 100-500 μm diameter.

According to a fourth aspect (fourth separation method) of the present invention, there is provided a method for separating collagen comprising vibrating a piece of fat which is extracted from a human body with an ultrasonic vibrator to destroy the fat cells, and separating collagen from a fat component which has a smaller specific gravity than that of the collagen.

Preferably, the ultrasonic vibration is effected by an electrical ultrasonic vibrator.

This invention will be described below with reference to the accompanying drawings, in which;

Fig.1 is a enlarged plan view of a filter which is used in the third separation method according to the present invention; and

Fig.2 is a schematic view of an ultrasonic vibrator which is used in the fourth separation method according to the present invention.

Preferred enbodiments of the invention are as follows.

First Separation Method

In the first separation method, distilled water is used to separate collagen from fat.

Namely, a piece of fat which is extracted from a human body by a known surgical means is pulverized into gruel form by a stirrer (homogenizer). Thereafter, distilled water is added to the gruel-like pulverized fat piece. A fat cell is composed by an outer shell which is mainly made of collagen and a fat component surrounded by the outer shell. The addition of distilled water causes the fat cell to be completely destroyed by the osmotic pressure, so that the collagen and the fat component are separated from one another. In this state, however, the fat cells including the collagen and the fat components look like orange juice.

Thereafter, the liquid component is introduced into a centrifugal separator in which the liquid component is divided into a jellied collagen having a large specific gravity and an oily fat component having a small specific gravity. The separated collagen is taken out by an autopipette or the like.

The collagen (autocollagen) obtained by this method can be directly used as a collagen to be injected into the person himself or herself. It is also

possible to freeze the collagen for a long time storage. The frozen collagen can be defrosted to be used.

Second Separation Method

In this method, the collagen is separated from fat by a quick freezing.

Namely, a piece of fat which is extracted from a human body by a known surgical means, similar to the first separation method, is mixed with distilled water, so that the fat cells expand owing to the osmotic pressure of the distilled water.

Thereafter, the fat cells are quickly frozen to a temperature below -50°C. Consequently, the water component of the fat cells is frozen, so that a further expansion of the fat cells takes place, thus resulting in a complete destruction of the fat cells.

When the frozen fat cells are defrosted, a liquid product (semi-product) including the collagen and the fat component mixed therewith can be obtained.

Thereafter, the liquid semi-product is divided into a jellied collagen having a large specific gravity and an oily fat component having a small specific gravity by a centrifugal separator. The separated collagen is taken out by an autopipette or the like.

In this method, the frozen fat cells which are obtained in the freezing process mentioned above can be stored for a long time and can be defrosted and separated into the collagen and the fat component when used.

Third Separation Method

In the third method, the collagen is separated from fat by filtering.

Namely, a piece of fat is extracted from a human body by a known surgical means, so that a small quantity of distilled water is added to the extracted fat piece. The addition of distilled water causes a fat cell which is composed of an outer shell which is mainly made of collagen and a fat component surrounded by the outer shell, as mentioned before, to be completely destroyed by the osmotic pressure, so that the collagen and the fat component are separated from one another. In this state, however, the fat cells including the collagen and the fat component look like orange juice. Preferably, the quantity of distilled water is about 20% relative to the amount of the fat cells.

In the third embodiment, the fat is extracted into a fat extracting injector (10 cm^3) in which about 2 cm^3 of distilled water is contained in advance.

Thereafter, the liquid semi-product including the collagen and the fat component is filtered to quickly separate a fibered and jellied collagen and an oily fat component. As shown in Fig. 1, the size of the openings 11 of the filter 10 used in this method is such that the fibered collagen can be effectively separated, and preferably is 100-500 μm. A filter to be advantageously used is a polyester filtering net which is usually used in a blood transfusion instrument and which has about 100 μm diameter of net wires and about 200 μm diameter of the openings.

Upon filtering, a slight hydraulic pressure is preferably applied. In the embodiment, the fat component having distilled water mixed therewith is delivered with pressure from the injector.

The collagen which is separated and maintained in the filter is taken out.

The collagen (autocollagen) obtained by the third separation method can be directly used as collagen to be injected into the person himself or herself. It is also possible to freeze the collagen for a long time storage. The frozen collagen can be defrosted to be used.

Fourth Separation Method

In the fourth method the collagen is separated from fat by ultrasonic vibration.

Namely, a piece of fat which is extracted from a human body by a known surgical means is introduced into an electrical ultrasonic generator. As the electrical ultrasonic generator used in this embodiment can be used an ultrasonic cell crushing device, such as a "SONIFIER" which is available on the market by Branson Co. Ltd. in the United States which is used to destroy or crush an animal cell or plant tissue, to destroy bacteria or yeast, to cut DNA chain or to separate virus of DNA cells, etc.

As shown in Fig.2, the ultrasonic cell crushing device 20 has a converter which converts electrical energy to longitudinal mechanical vibration. When a tip 22 of the front end of a horn 21 of the device 20 is put in a solution, the mechanical vibration is transmitted to the solution as a pressure wave to cause cavitation. Namely, the tip of the horn put in the fat solution produces a pressure wave in the solution, so that air bubbles are produced by local negative pressure at the negative cycle of pressure wave. The air bubbles are pressed and collapsed at the positive cycle of pressure wave (This is called cavitation). The fat cells are destroyed by the strong impact of the repeated formation and destruction of the air bubbles in the solution.

The fat cells are thus completely destroyed by the ultrasonic vibration, so that the oil (fat) component and the collagen are separated from one another. In this state, however, the fat cells including the collagen and the fat component look like orange juice.

Thereafter, the liquid component is introduced into a centrifugal separator in which the liquid component is divided into a jellied collagen having a large specific gravity and an oily fat component having a small specific gravity. The separated collagen is taken out by an autopipette or the like.

The collagen (autocollagen) obtained by this method can be directly used as a collagen to be injected into the person himself or herself. It is also possible to freeze the collagen for a long time storage. The frozen collagen can be defrosted to be used.

As can be understood from the foregoing, according to the present invention, the collagen which is extracted from a patient himself or herself can be injected as so called "autocollagen" into his or her own body. Namely, the wrinkled skin or the skin with depressions, such as a scar, or depressions caused by pimple can be flattened or remedied by the collagen which is directly injected into a patient's dermis of the senile lines (wrinkles) or depressions by an injector without an allergic reaction. The removal of such senile lines causes the patient to expect to have a rejuvenated skin.

Since the collagen has a moisture effect in which an oil component and moisture of a skin is maintained on the skin surface, the collagen can be used as a cosmetic cream, a cosmetic latex, or other cosmetics to maintain the moisture of the skin. In this case, the autocollagen obtained by the present invention can be used as a high quality cosmetic product for himself or herself. As mentioned before, in case of an autocollagen, no allergic reaction occurs.

As can be seen from the above discussion, according to the present invention, it is possible to easily and effectively separate collagen from fat. The separated collagen, if frozen, can be stored for a long period and can be used anytime by defrosting the same.

Claims

1. A method for separating collagen comprising;
   adding distilled water to a piece of fat which is extracted from a human body, so that the fat cells are destroyed by the osmotic pressure of the distilled water; and,
   separating collagen from a fat component which has a smaller specific gravity than that of the collagen.

2. A separation method according to claim 1, wherein the separation of the collagen from the fat component is effected by a centrifugal separation.

3. A method for separating collagen comprising;
   adding distilled water to a piece of fat which is extracted from a human body;
   quickly freezing the mixture of the fat piece and the distilled water to a temperature below -50°c to destroy the fat cells; and thereafter,
   separating collagen from a fat component which has a smaller specific gravity than that of the collagen.

4. A separation method according to claim 3, wherein the separation of the collagen from the fat component is effected by a centrifugal separation.

5. A method for separating collagen comprising;
   adding a small amount of distilled water to a piece of fat which is extracted from a human body; and,
   filtering the mixture of the fat piece and the distilled water to separate collagen from the fat cells.

6. A separation method according to claim 5, wherein the separation of the collagen from the fat cells is effected by a filter which has a large number of openings of 100-500 μm diameter.

7. A method for separating collagen comprising;
   vibrating a piece of fat which is extracted from a human body by an ultrasonic vibrator to destroy the fat cells; and,
   separating collagen from a fat component which has a smaller specific gravity than that of the collagen.

8. A separation method according to claim 7, wherein the ultrasonic vibration is effected by an electrical ultrasonic vibrator which converts electrical energy to a mechanical ultrasonic vibration.

9. A separation method according to claim 7, wherein the separation of the collagen from the fat component is effected by a centrifugal separation.

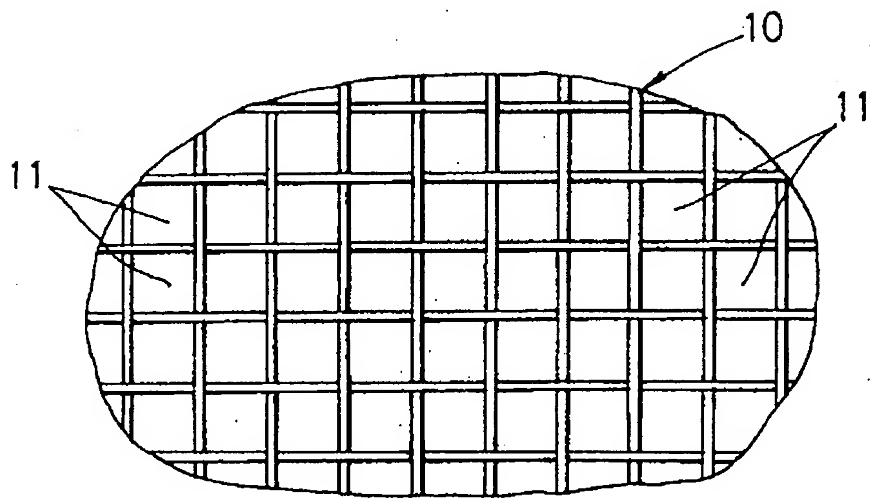

FIG-1

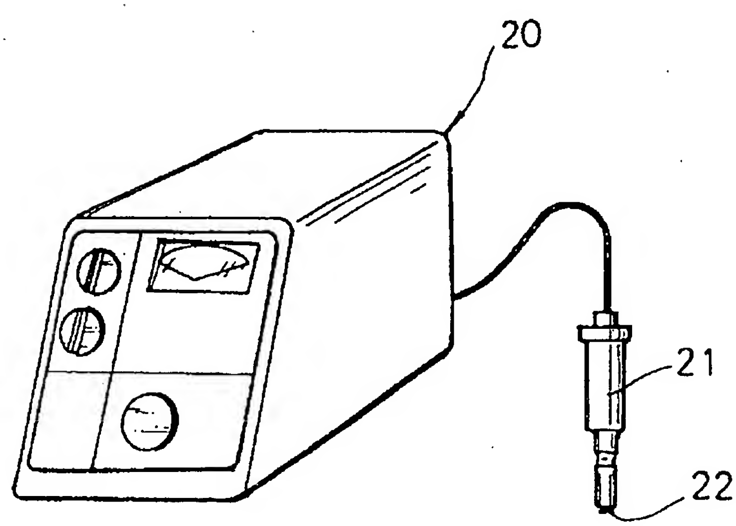

FIG-2

European
Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 11 3376

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 284 789 (ISTITUTO GENTILI) * Whole document, especially example 1; column 4, lines 25-55 * – – –	1-6	C 07 K 15/00 C 08 H 1/06 A 61 L 27/00
A	EP-A-0 330 389 (KELMAN) * Example 1; page 11, lines 25-46 * – – –	1-6	
A	DE-A-1 936 957 (SCHALLER et al.) * Page 2, lines 6-33 * – – –	1-6	
A	CHEMICAL ABSTRACTS, vol. 74, no. 7, 15th February 1971, page 157, abstract no. 29867b, Columbus, Ohio, US; M.G. STEELE et al.: "Age changes in the subunit composition of rat tail tendon collagen extracted at 65 degrees in water and at 2 degrees in acid", & GERONTOLOGIA 1970, 16(5), 277-82 * Abstract * – – –	1-6	
E	EP-A-0 418 979 (ZOCCHI) * Whole document * – – – – –	7-9	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 K
C 08 H
A 61 L

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	06 July 91	MASTURZO P.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document

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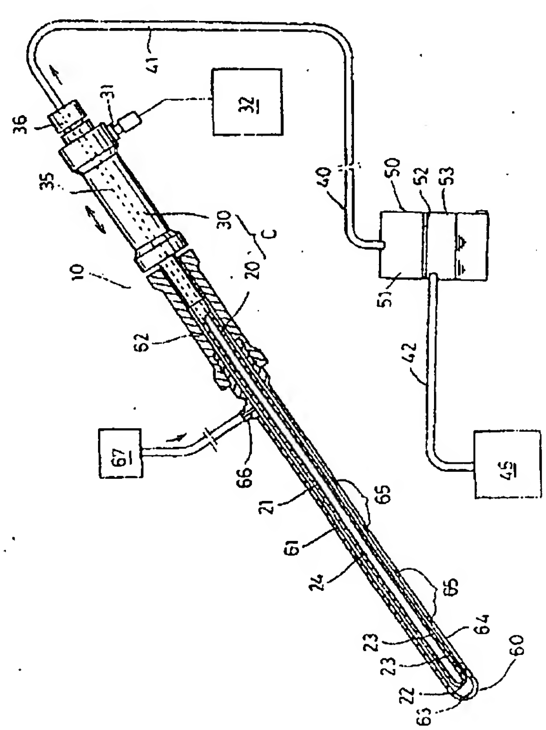
(54) Collagen gathering apparatus.

(57) A collagen gathering apparatus including a fat tissue crusher (10) having a cannula which is inserted in a fat tissue to crush the fat tissue by an ultrasonic oscillation, a sucker having a suction pipe (21) and a vacuum pump (45) for sucking the fat tissue crushed by the crusher, and a collector (50) which is connected to the suction pipe (41) of the sucker and which has a filter (52) for separating the fat tissue into a collagen and a liquid component.

Fig. 1



EP 0 515 726 A1

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an apparatus for gathering collagen, and more precisely, it relates to an apparatus for crushing and sucking a fat tissue and separating it into collagen and liquid component.

2. Description of Related Art

For instance, in order to remove depressions, such as senile wrinkles or scars in a cosmetic surgery, it is known to directly inject collagen into his or her skin, using an injector, so that the depressions can be flattened. The collagen is made of fibrous protein which is a main component of a combined tissue which makes up a bone or skin, etc.

In a conventional cosmetic surgery, collagen is usually extracted from a cowhide and refined. However, there is a possibility of an occurrence of an allergic reaction due to heterogeneousness of the collagen (protein) from the human body.

To eliminate the problem with the allergic reaction, the inventors of the present invention have focused on the recent cosmetic surgery in which the fat is extracted from the buttocks or the belly and have proposed in Japanese patent Application No. 2-118016 that the collagen is extracted from the extracted fat and injected into his or her own skin. The collagen which is extracted from his or her own fat tissue is free from the allergic reaction due to heterogeneous protein.

In a conventional method for extracting the fat from the human body, a hollow tubular cannula is inserted into a subcutaneous tissue and is moved in the subcutaneous tissue to scrape the fat which is then sucked outward by a suction pump or the like through a passage formed in the cannula.

However, in the known method, there are drawbacks as follows:

(a) it is necessary for an operator to carefully and troublesomely move the cannula in the subcutaneous tissue which is mechanically crushed or destroyed by the cannula to scrape the fat from the subcutaneous tissue;

(b) it is difficult to precisely scrape the fat at a predetermined portion of the subcutaneous tissue by the cannula which is manually moved by an operator, thus resulting in a decrease in scientific reliability;

(c) there is a large possibility that a soft tissue other than fat can be injured by the movement of the cannula upon scraping the fat; and,

(d) it is particularly difficult to control the bleeding from an cannula insertion portion of the human body and the soft tissue, injured by the cannula.

The primary object of the present invention is to eliminate the drawbacks mentioned above by providing a collagen gathering apparatus in which the fat can be effectively extracted from the human body and the collagen can be simply and effectively separated from the fat thus extracted.

Another object of the present invention is to provide a collagen gathering apparatus in which the fat can be precisely and simply taken out from a predetermined portion of the subcutaneous tissue by an operator to prevent the soft tissue other than the fat from being injured and to extremely decrease the bleeding.

SUMMARY OF THE INVENTION

To achieve the object of the present invention as mentioned above, there is provided a collagen gathering apparatus comprising a fat tissue crusher having a cannula which is inserted in a fat tissue to crush the fat tissue by an ultrasonic oscillation, a sucker having a suction pipe and a vacuum pump for sucking the fat tissue crushed by the crusher, and a collagen collector which is connected to the suction pipe of the sucker and which has a filter for separating the fat tissue into a collagen and a liquid component.

Brief Description of the Drawings

The invention will be described below in detail with reference to the accompanying drawings, in which:

Figure 1 is a partially sectioned side elevational view of a collagen gathering apparatus according to an aspect of the present invention;

Fig. 2 is a schematic bottom view of a tip and an outer tube of a collagen gathering apparatus shown in Fig. 1;

Fig. 3 is a sectional view of a collector according to a first embodiment of the present invention; and,

Fig. 4 is a sectional view of a collector according to another embodiment of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As can be seen from Fig. 1, the collagen gathering apparatus of the present invention basically includes a fat tissue crusher 10, a sucker 40 and a collector 50.

The fat tissue crusher 10 has a cannula 20 which is comprised of a tip 20 and a hand piece 30 integral therewith. The tip 20 has a tubular body 21

of metal, such as titanium. The tip 20 is provided with a rounded front end 22 and sucking openings 23, 23 on the lower surface of the tube in the vicinity of the front end 22, as can be seen in Fig. 2. The tubular body 21 defines therein a suction passage 24 of the collagen which is crushed and extracted. In the illustrated embodiment, the tip 20 has 5 mm diameter and about 500 mm length.

The tip 20 is slidably inserted and supported in an outer tube 60. The outer tube 60 is made of light plastics having a high heat-resistance, such as fluoroplastics or the like. The outer tube 60 is comprised of a tubular body 61 which is inserted in the subcutaneous tissue and a grip portion 62 which is held by an operator.

The tubular body 61 protects the soft tissue from the movement of the tip 20 and heat due to the ultrasonic oscillation. The tubular body 61 also contributes to a precise removal of a predetermined fat tissue from the human body. The front end 63 of the tubular body 61 is rounded to prevent the skin and the subcutaneous tissue from being injured upon insertion of the tubular body. The tubular body 61 is provided on the lower surface thereof with an appropriate number of sucking openings 64 aligned along the length thereof, as shown in Fig. 2. Numeral 65 designates reinforcing bridge portions between the sucking openings 64. In the illustrated embodiment, the tubular body 61 has 10 mm diameter and about 340 mm length.

As can be seen in Fig. 1, the tubular body 61 is provided on its base end with a water supplying port 66 through which a coolant is fed from a coolant source 67 into the tubular body 61 to cool the heat produced in the tip 20, in accordance with need. The coolant is sucked together with the crushed fat. For example, a balanced saline solution or the like can be used as a coolant.

The tubular body 61 is screwed in and connected to the front end of the grip 62. The whole length of the outer tube 60 having the grip 62 and the tubular body 61 attached to the grip 62 is substantially equal to or slightly longer than the length of the tip 20.

The hand piece 30 is held by an operator to manually actuate the apparatus. The tip 20 is detachably connected to the front end of the hand piece 30. The hand piece 30 has an oscillation generating portion 31 and a suction passage 35.

The oscillation generating portion 31 is connected to a ultrasonic oscillator 32, so that the electric energy from the oscillator 32 is converted to the ultrasonic oscillation which is then transmitted to the tip 20 attached to the hand piece 30.

For instance, in the illustrated embodiment, the oscillation generating portion 31 generates the ultrasonic oscillation of 300 μ m amplitude and 24000/sec frequency.

The ultrasonic oscillator 32 supplies the oscillation generating portion 31 of the hand piece 30 with electric energy which can be converted to the ultrasonic oscillation. In the illustrated embodiment, the ultrasonic oscillator 32 has an electrostrictive strain vibrator PZT of 24 kHZ of frequency of vibration and 100 W of maximum output.

The suction passage 35 is connected at its front end to the suction passage 24 of the tip 20 and at the rear end thereof to a front suction pipe 41 of the sucker 40, respectively. Numeral 36 designates a connecting portion of the suction pipe 41.

The sucker 40 has the front suction pipe 41 and a rear suction pipe 42, and a vacuum pump 45. The front suction pipe 41 is connected to the suction passage 35 of the hand piece 30, as mentioned above, so that the crushed fat tissue sucked from the sucking openings 23 of the tip 20 is forcedly sucked into the collector 50 by the vacuum pump 45 connected to the rear suction pipe 42.

The collector 50 is located between the front and rear suction pipes 41 and 42 of the sucker 40, as shown in Fig. 3. The collector 50 has a body casing 51 and a filter 52 in the body casing 51. The crushed and sucked fat tissue is introduced in the body casing 51 and separated into the collagen K and the liquid component L by the filter 52. The filter 52 is held on and by a flange portion 55 of the body casing 51. The body casing 51 has an inlet port 56 connected to the front suction pipe 41 and an outlet port 57 connected to the vacuum pump 45 through the rear suction pipe 42.

The filter 52 has a filtering efficiency large enough to separate the collagen K of the fibrous component from the crushed fat tissue and can be realized, for example by a filtering film of polyester or nylon of about 100 ~ 500 μ of opening diameter and 50 ~ 100 μ of wire diameter, used in a known blood transfusion instrument.

The crushed fat tissue passing through the suction pipe 41 is rapidly separated into the liquid components L, such as water, oil, blood, lymph, etc., and the collagen K of fibrous component by the filter 52 of the collector 50. The liquid components L pass through the filter 52 and are collected in a reservoir 53 defined by the lower portion of the body casing 51. The collagen K is collected on the filter 52. Numeral 59 designates a liquid discharging port.

Figure 4 shows a modified collector 70. In this embodiment, the crushed fat tissue passing through the suction pipe 41 is introduced into a tubular casing 71 through an inlet port 76, so that the collagen K is separated and collected from the fat tissue by the filter 72 provided at the outlet end (lower end) of the tubular casing 71. The liquid components past the filter 72 are collected in a

reservoir (not shown) connected to the suction port 77 of the tubular casing 71.

The collagen thus obtained can be directly used as an autocollagen to himself or herself. It is possible to freeze the collagen for a long preservation, so that the frozen collagen can be defrosted to be used, in accordance with need.

As can be understood from the foregoing, according to the present invention, the fat tissue is directly crushed by the ultrasonic oscillation of the fat tissue crusher, and is sucked by the sucker, so that the collagen is separated from the fat tissue and collected in the collector.

According to the invention, since the fat tissue is directly crushed by the ultrasonic oscillation of the crusher, the crushed fat is extracted as it were melted butter. Consequently, the collagen can be simply and easily separated and collected from the crushed and extracted fat tissue only by the filter without using a special separating apparatus, such as a centrifugal separator.

Furthermore, according to the present invention, since no mechanical crush or destruction of the fat tissue takes place, unlike the prior art, the bleeding can be controlled to be minimized.

In addition, the labor of the operator can be largely decreased, and the fat tissue can be precisely picked at a desired portion of the subcutaneous tissue.

Furthermore, since the intensity and frequency of the ultrasonic wave of the ultrasonic oscillator can be simply and easily controlled, the necessary ultrasonic oscillation can be easily obtained.

Claims

1. A collagen gathering apparatus comprising:

   a fat tissue crusher having a cannula which is inserted in a fat tissue to crush the fat tissue by an ultrasonic oscillation;

   a sucker having a suction pipe and a vacuum pump for sucking the fat tissue crushed by the crusher; and,

   a collagen collector which is connected to the suction pipe of the sucker and which has a filter for separating the fat tissue into a collagen and a liquid component.

2. A collagen gathering apparatus according to claim 1, wherein said cannula comprises a tip having a tubular body which defines therein a suction passage and which has at least one suction opening at the front end of the tubular body, and a hand piece to which the tip is detachably connected and which has therein a suction passage which is connected to the suction passage of the tip.

3. A collagen gathering apparatus according to claim 2, wherein said hand piece further comprises an ultrasonic oscillation generating portion which transmits the ultrasonic oscillation to the tip.

4. A collagen gathering apparatus according to claim 3, further comprising an ultrasonic oscillation source connected to the ultrasonic oscillation generating portion.

5. A collagen gathering apparatus according to claim 2, further comprising an outer tube in which the tip can be slidably inserted and held.

6. A collagen gathering apparatus according to claim 5, wherein said outer tube is provided with at least one suction opening.

7. A collagen gathering apparatus according to claim 4, wherein said suction pipe comprises a front suction pipe which is connected to the suction passages of the tip and hand piece and a rear suction pipe which is connected to the front suction pipe and the vacuum pump.

8. A collagen gathering apparatus according to claim 7, wherein said collector is located between the front and rear suction pipes.

9. A collagen gathering apparatus according to claim 8, further comprising a reservoir provided in the collector, so that the liquid component separated from the fat tissue can be collected in the reservoir.

10. A collagen gathering apparatus according to claim 9, wherein the collagen separated from the fat tissue is collected by and on the filter.

11. A collagen gathering apparatus according to claim 2, further comprising a cooler for cooling the tip.

12. A collagen gathering apparatus comprising:

    an ultrasonic oscillation crushing means, inserted in a fat tissue of a human body for crushing the fat tissue with an ultrasonic oscillation;

    a sucking means for sucking the fat tissue thus crushed;

    a passage means for conveying the sucked fat tissue therethrough;

    a collecting means for collecting the fat tissue conveyed through the passage means; and,

    a separating means for separating a collagen from the collected fat tissue.

13. A collagen gathering apparatus according to claim 12, further comprising an ultrasonic oscillation generating means for generating the ultrasonic oscillation.

14. A collagen gathering apparatus according to claim 13, further comprising a cooling means for cooling the apparatus.

Fig. 1

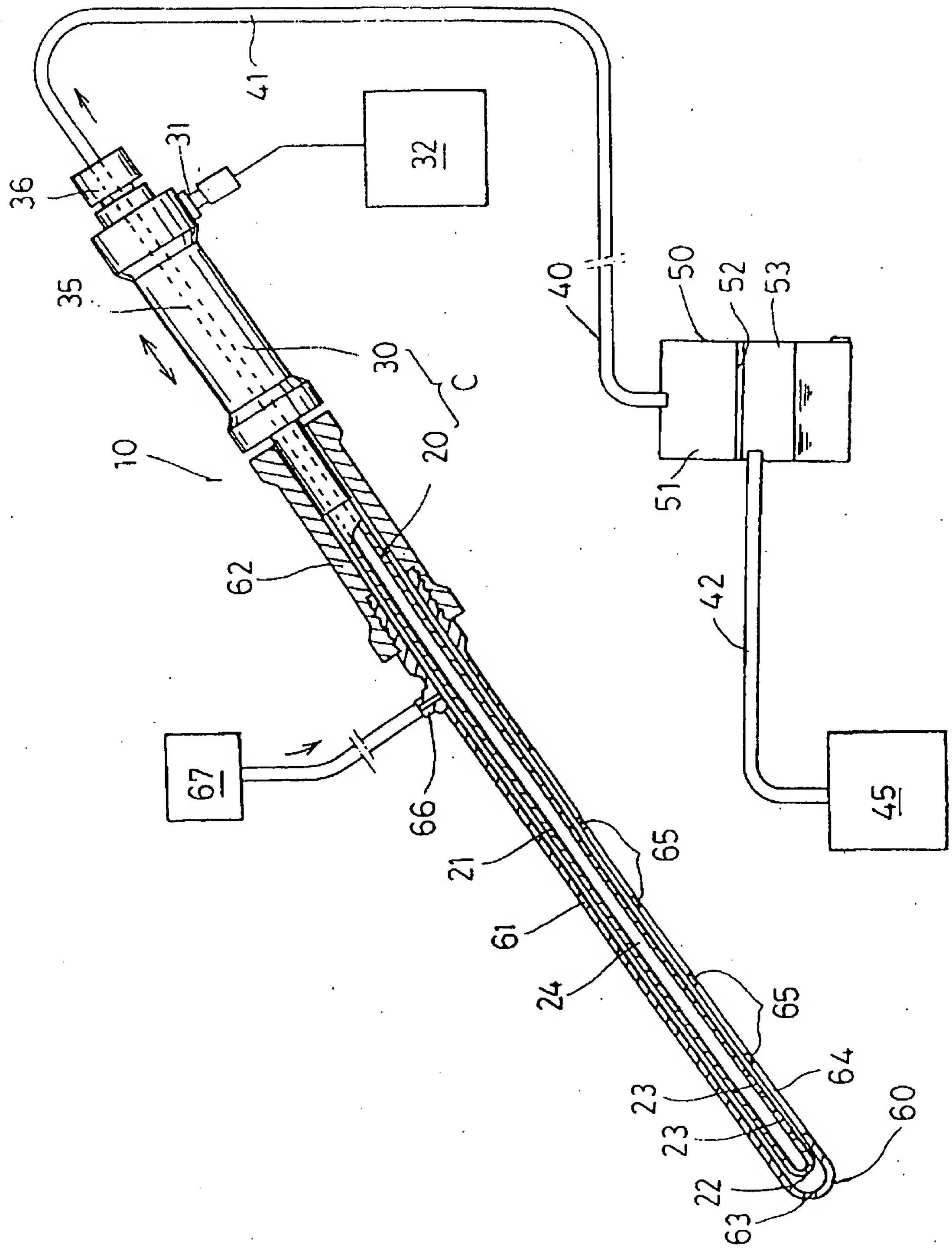

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Fig. 2

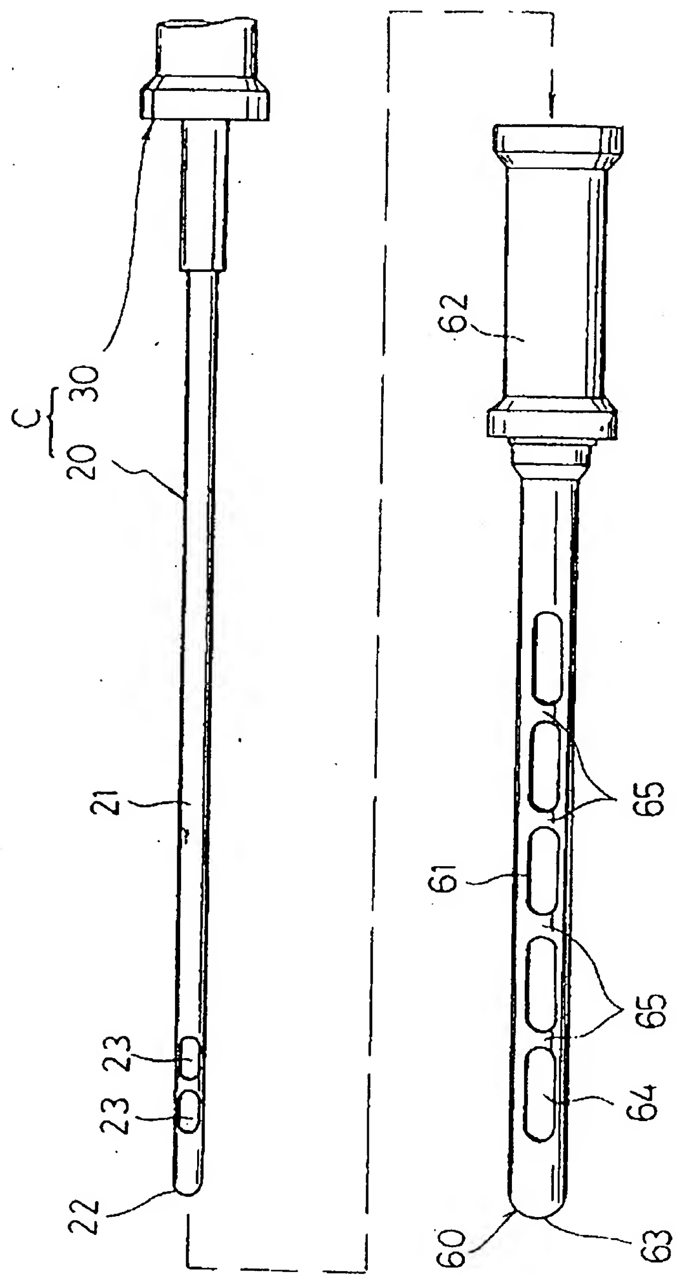


Fig. 3

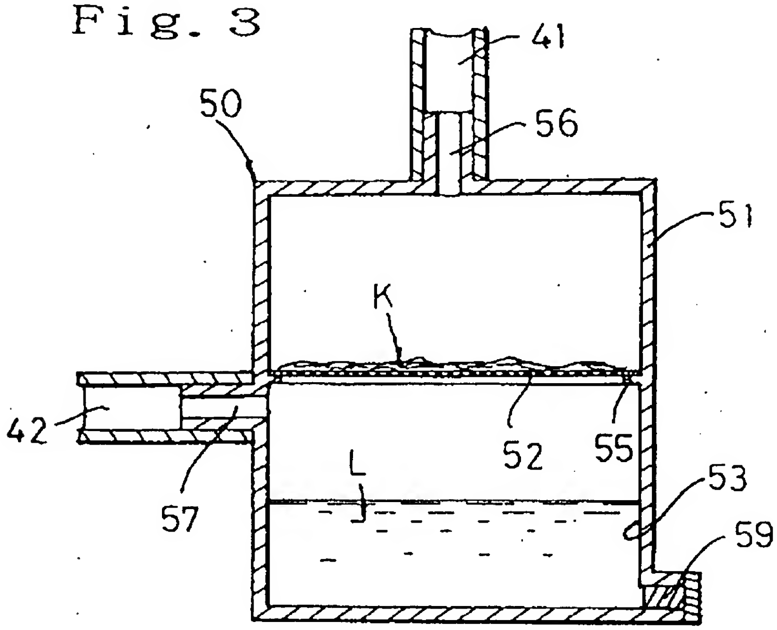
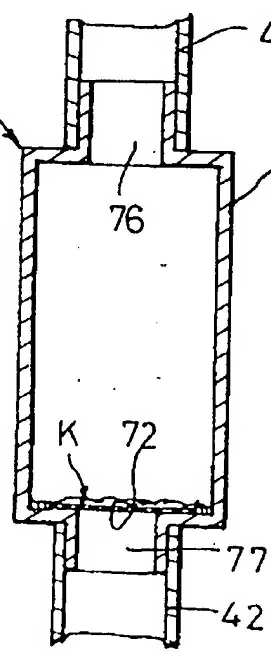


Fig. 4



European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 11 4583

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	EP-A-0 331 313 (T PARISI) * the whole document * ---	1-4,7-14	A61B17/22 A61M1/00
A	EP-A-0 346 496 (SUMITOMO BAKELITE CY LTD) * page 5, line 8 - page 6, line 16 * * page 7, line 1 - line 7 * * page 10, line 6 - line 11 * * figures 1-3 * ---	1-4,7-14	
Y	US-A-4 710 162 (G JOHNSON) * column 1, line 61 - line 66; figures 1-3 * ---	1-4,7-14	
A	WO-A-8 602 844 (STRYKER CORP.) * page 11, column 5 - page 13, column 4 * * figure 2 * ---	1,8-10	
A	US-A-4 834 703 (W DUBRUL) -----		
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			A61B A61M

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	03 SEPTEMBER 1992	VEREECKE A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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FAT GRAFTING

James E. Fulton, MD, PhD, and Noushin Parastouk, MD

Successful tissue augmentation remains difficult. Before 1992, silicone was useful for filling small deficits, and bovine collagen was used for larger augmentations. Silicone is no longer readily available in the United States, and bovine collagen is a temporary augmentation that occasionally results in allergic reactions. The possible use of the patient's own tissue has always been a favorite option. However, there have been technical problems obtaining this material and maintaining its retention.

The use of one's own adipose tissue has been a recurring theme since the article by Neuber[7] in 1893. He used fat from the back of the arm to fill facial tissue defects resulting from tuberculosis. Articles by Peer[11,12] rekindled interest in the 1950s. He demonstrated an average retention of 40%–50%. Interest flourished in the 1980s with the development of liposuction because of the easy availability of tissue.[8] Askin[1] demonstrated that the thigh was a preferential donor site. Nguyen et al[9] suggested that muscle was the best recipient site. Coleman[3] concentrated fat by centrifugation and used this fat in multiple planes across the face for a facelift bypass. In 1994, Carpaneta[2] published graft viability studies indicating the injected volume of adipose tissue should have a diameter of no more than 3 mm. We have taken these suggestions along with our own experiences[5] to develop guidelines for fat transfer.

METHODS AND MATERIALS

Indication

The use of fat for tissue augmentation is indicated in any subcutaneous location where there is atrophy or loss of tissue. Fatty tissue is useful for replacing buccal fat pads, divots after liposuction, and scar depressions, especially when combined with subcision.[10] Subcision is important to lyse the fibrous-connecting bands so the fat can be layered between the dermal tissue and the underlying fascia in areas such as acne scars or traumatic scars. Adipose tissue is also effective in augmenting natural features over bone such as chin implants, malar implants, and jawline augmentation. Fat is useful to reverse areas of atrophy such as loss of turgor of the lips and back of hands. In addition, areas of atrophy following steroid injections or diseases such as linear morphia or lupus panniculitis can be improved by subcision and adipose tissue infiltration.

Informed Consent

A complete consultation outlining the benefits and risks of the procedure, the benefits

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and risks of alternatives, and answering all the patient's questions is imperative. It is particularly important with adipose-tissue augmentations to explain that it may require two or three sessions to achieve complete patient satisfaction. The patient needs to understand that the defect will be overcorrected 20%–30%. Because the augmentation is often with thigh fat, the area may increase in size in some cases with weight gain, necessitating some removal. Bruising and secondary infections are also discussed with the patient, so the patient discontinues aspirin, vitamin E, and ibuprofen. Patients also take an antibiotic for 7 to 10 days after the procedure.

Proper patient selection is imperative. The patient's expectations must be realistic. Modest augmentations are possible over cheekbones, chin, and lips; however, if the patient is looking for a more dramatic appearance, a hard implant may be more useful. The patient must also have an adequate donor site and not be too thin with no fat. Those with adequate fat stores do better than thin patients who have very limited fat.

Donor Sites

We prefer the outer thigh. This fat, especially in female patients, is resistant to weight loss, has low levels of vascular profusion, and produces an excellent fat harvest with minimal trauma. Usually, this outer thigh area is approached through the lower buttock crease, which leaves minimal visible scar. Patients wash the donor site and the recipient site the night before and the morning of the procedure with chlorhexidine (Hibiclens®). After cleansing the donor port with povidone-iodine, the site is anesthetized with lidocaine with epinephrine (lidocaine 2% with epinephrine 1:200,000). A small stab incision is made with a 15-c blade in the buttock crease area. Tumescent fluid is infiltrated into the donor recipient site (List 1). Usually 2–3 mL is infiltrated for each mL that is to be removed. After waiting for 15 minutes for vasoconstriction, fat from the donor site is harvested with a 2.5-mm Mercedes tip cannula attached to a "vented" 20-mL syringe (Fig. 1). The venting is accomplished by producing a 2-mL air pocket in the syringe. Gentle vacuum is applied with the plunger as the needle transverses gently through the fatty area. Usually, four or five 20-mL syringes are filled with the combination of this tumescent fluid and adipose tissue. If the fat is blood tinged, it is washed once or twice with lactated Ringer's solution before centrifugation at 3,000 rpm for three minutes. If a firmer implant is desired, bone powder (1% to 10%) can be added (Demineralized Cortical Bone Powder, American Red Cross, Costa Mesa, CA) (Fig. 2). After mixing the fat and bone powder with manual hand agitation in the sieve, the adipose tissue is transferred to 3-mL syringes for injection. Meanwhile, the donor site is bound with support garments. The unsutured donor port drains for several days, and the support garment is worn for 2 weeks.

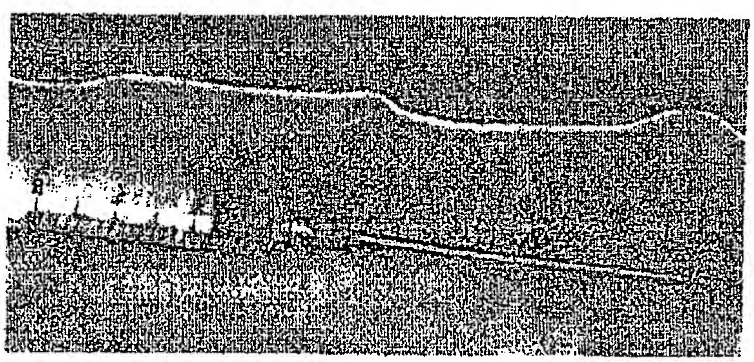

Figure 1. Vented syringe. Fat is collected with a vented 20-mL syringe using a 2.5-mm cannulae with a Mercedes tip. The addition of air maintains the liposuction at a reduced negative pressure.

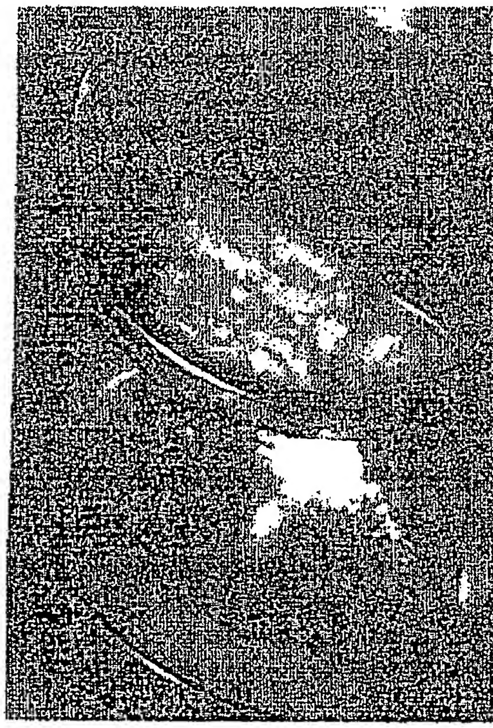

Figure 2. The sieve. The excessive tumescent fluid is released through the sieve. Bone powder (1% to 10%) can be added at this point to produce a firmer implant

List 1. TUMESCENT SOLUTION

Lactated ringers	500 cc
Lidocaine 1%	50 cc
Epinephrine (1:1000)	2 cc

Recipient Site

The recipient area is also cleansed with povidone-iodine. Injections of lidocaine with epinephrine are used to accentuate the actual port of injection along inconspicuous lines such as the hairline, commissures of the lips, or the natural crease under the jawline. Local anesthetic is then infiltrated along the injection track and close to the terminal injection site. After a waiting period for vasoconstriction, the fat is transferred with a 14-, 16-, or 18-gauge curved blunted needles (Fulton needle, KMI, Inc., Anaheim, CA). The fat is gently injected as small filaments on the retrograde pass of the needle as it is withdrawn from the recipient site. This fat is injected into multiple tissue planes; for example, in the chin, fat is injected first deep into the periosteal area, then into the muscle area, and finally into the subcutaneous tissue plane. Any resistant fibrous trabeculae can be subcised with this blunted needle tip. Care is taken to avoid bleeding because extravasated hemoglobin will induce an inflammatory response which may detrimentally affect the fat transfer. The desired augmentation is overcorrected by approximately 20%–30%. Except for the ports at the oral commissures, which are closed with 7-0 fast-absorbing gut, the skin injection sites are left open to drain. A protective dressing of tube-gauze is applied for 24 or 48 hours depending upon the area of augmentation. Patients apply ice compresses to the area twice daily. They also receive intramuscular injections of 9–12 mg betamethasone (Celestone®, Schering Corp., Kenilworth, NJ) and oral cephalexin (500 mg bid) for 7–10 days after the procedure. Additional fat is stored in 3-mL syringes in a dedicated freezer equipped with a temperature Sensaphone (Phonetics Inc., Aston, PA) at [illegible] C. If necessary, repeat augmentations are completed at 2-month intervals using this frozen tissue.

RESULTS

We reviewed our results of 300 injection sites on 145 cases between [illegible] and [illegible]. Most patients were quite satisfied with their soft-tissue augmentations (Figs. 3 and 4). Some patients required a second or third session to achieve a satisfactory augmentation. The use of allografts was not necessary. For the nasolabial folds, marionette lines, and glabellar lines, it was necessary to subcise the subcutaneous trabeculae in addition to the fat transfer to attain long-term results. The fibrous attachments of the skin to the fascia were lysed, and a layer of fat was placed between the skin and underlying tissue. This prevented the reattachment of the fibers and helped maintain a more permanent augmentation.

The buccal fat pad was one of the more satisfying areas for fat augmentation (Fig. [illegible]). This replaced the cadaveric appearance and generated a more youthful, round face. How

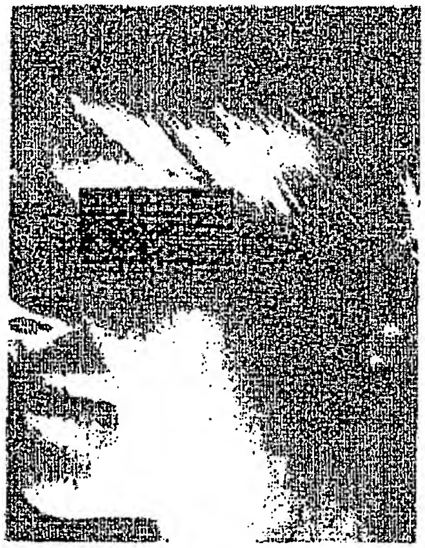

Figure 3. Cheekbones. A dramatic new profile can be achieved with bone powder (10%) mixed with adipose tissue.

ever, this augmentation was so successful that over the years a few patients developed too much buccal fat, and some fat had to be removed with liposuction (Fig. 6). The lips were the most popular site for tissue augmentation but one of the more difficult areas to achieve lasting benefits (Fig. 7). The percent of retention was less than over bony protuberances such as the cheekbones and chin and often required a second or third session to achieve patient satisfaction.

The dorsal aspect of the hand was another successful area for augmentation (Fig. 8). The atrophic tissue was puffed out with the subdermal injection of 12–16 mL of fat. To protect the augmentation, foam and tube gauze was used as a dressing for 5 to 7 days after the procedure. A dusky blue appearance remained for several weeks until the fat became vascularized. Touch-ups were often required to develop a more uniform augmentation. Depressed areas such as liposuction divots or scars were also filled with autologous fat tissue. This retention was satisfactory and only seldom required a second session (Fig. 9).

Complications of this autologous fat transfer were minimal. The biggest problem was either the reabsorption of fat or the development of excessive tissue. This either required more augmentation or liposuction to remove it. Minor areas of bruising, persistent erythema, or a dusky gray appearance could remain for several weeks. This gradually disappeared as vascularization developed. A fibrous tumor similar to a lipoma developed occasionally in lower eyelid augmentations.

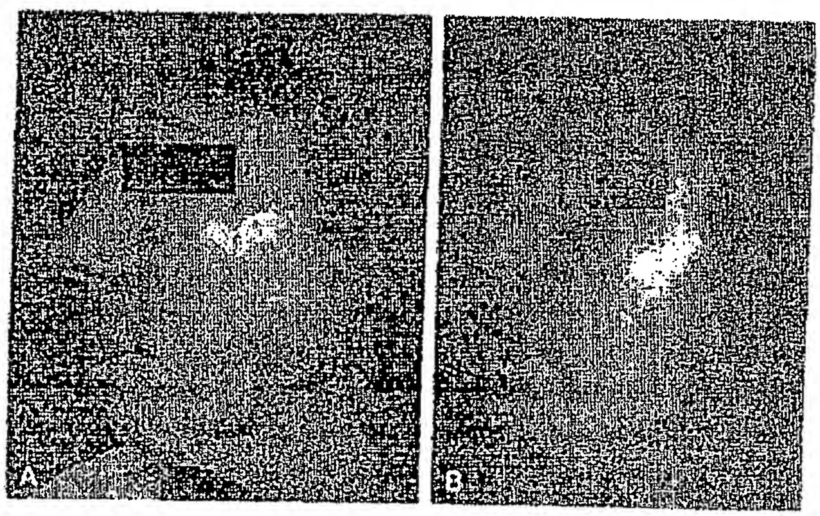

Figure 4. *A* and *B*, Chin augmentation. Adipose tissue and bone powder (10%) is injected into multiple planes (periosteal, muscle, and subcutaneous), providing a pleasing new profile.

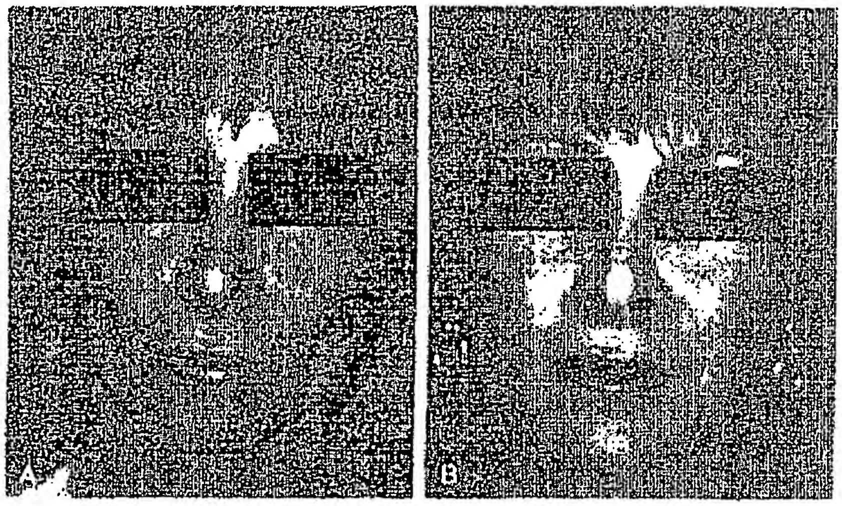

Figure 5. *A* and *B*. Buccal fat pad augmentation. Sixteen to 20 mL of adipose tissue is injected in multilayers to fill out the cheeks. This results in a dramatic rejuvenation.

and in the back of the hand. These were surgically removed. Infections developed at the injection site in three cases. The cultures demonstrated Staphylococcus or Streptococcus growth. When the patients were placed on the appropriate antibiotic and the area drained, the infection rapidly cleared. These patients were reinjected at a later date to complete their augmentation. After 90 days, the graft volume remained stable. The best areas for fat augmentation were the chin, the cheek bones, and the buccal fat pads. Augmentations to the lips and the nasolabial fold were less successful (Table 1).

Figure 6. Overcorrection. With time and weight gain, the augmentation may become excessive and require partial removal.

DISCUSSION

Fat grafting is now a safe and effective procedure. It is quite useful for [illegible] enhancing body contours. In fact, several patients developed retentions that were greater than 100% because the body picks up weight with time and the fatty deposits enlarged.

Table 1. PERCENT OF FAT RET[illegible]

Area	Volume (cc)	Retention†	Notes
Glabellar crease*	1-2		[illegible]
Crow's feet	3-6		With side effects
Nasolabial fold	3-4	[illegible]	With side effects
Marionette lines	2-3		[illegible]
Buccal fat pad	17-20	[illegible]	[illegible]
Eyelids	0.5-1		[illegible]
Angle of mouth [illegible]	[illegible] 16		[illegible]
Chin	[illegible] 12	[illegible]	[illegible]
Lips	[illegible]	[illegible]	[illegible]
Cheekbones	[illegible]		[illegible]
Hands	[illegible]	[illegible]	[illegible]

[illegible]

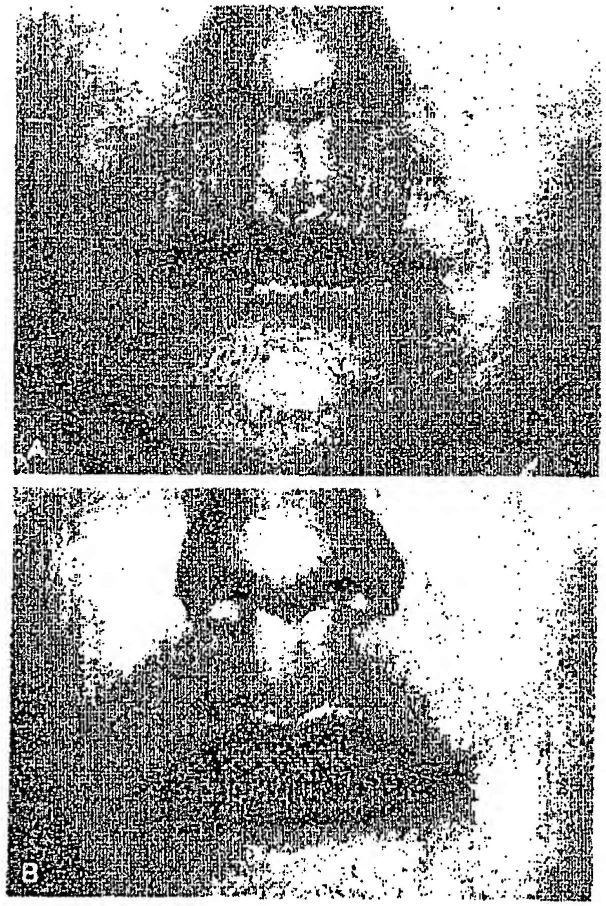

Figure 7. *A* and *B*, Lip augmentation. The combination of augmentation with fat and laser resurfacing produces a dramatic new lip.

Although any donor site with a substantial amount of fat can be used, the outer thigh proved the most efficacious. This site provided abundant fat, which was resistant to weight loss and was acclimated to low profusion rates. The fat could be harvested in a concentrated fashion without excessive bleeding. Other areas such as the inner thigh were less satisfactory because they yielded less fat and often became blood tinged. Although some surgeons prefer the inner knee, this area does not have adequate fat stores for large implants or multiple sessions.

The lower eyelids proved to be the most difficult areas to augment. Retention in the eyelid area was so good that overcorrections were often obtained. In two cases the fat had to be excised and removed. We found that eyelid augmentations were best done through a smaller needle with smaller volumes, into a deeper plane behind the muscle, and small repeat sessions to avoid over-augmentation.

The cheekbone and chin areas were excellent for fat retention. Injections were made into the periosteal plane, then the curved need was rotated into the subcutaneous plane, and finally filaments of fat were injected up into the area of the subdermis. This three-layer augmentation dramatized the cheekbones and chin.

Patients were extremely pleased with buccal fat pad augmentation. This is a simple procedure in which a small injection site below the jawbone can be used to inject the fat

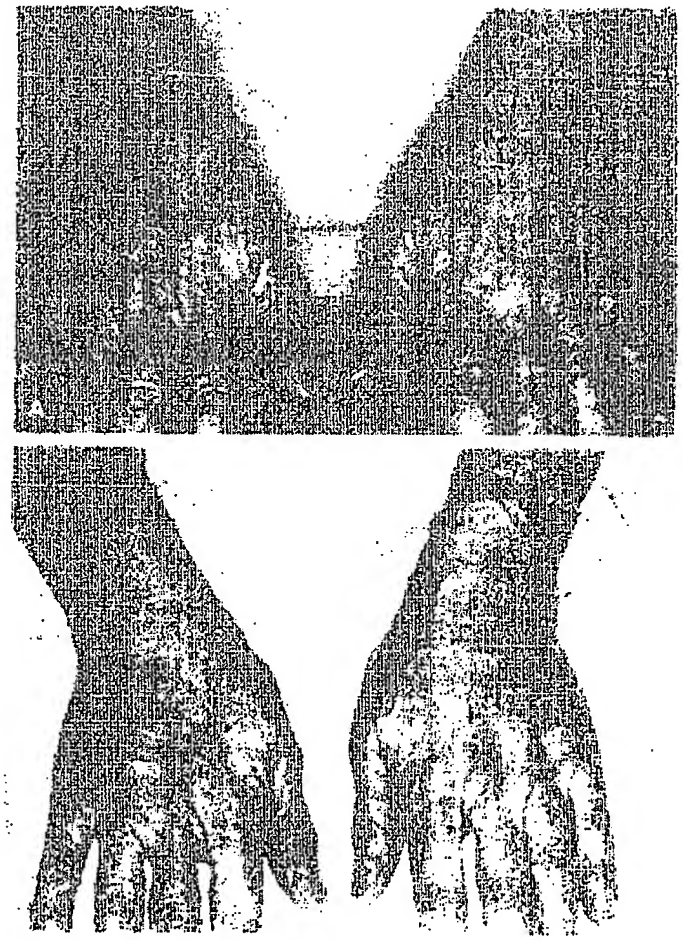

Figure 8. A and B The hands. The atrophic hands [illegible] fat augmentation and a chemical peel.

Figure 9. A and B Depressed [illegible] by placing fat into the [illegible]

in multiplanes into the buccal fat area. No excessive augmentation should be done because the fat is retained well and the augmentation may become too large with body weight gain and need to be partially removed.

Augmentations of the nasolabial fold were more difficult. The constant movement in this area resulted in less fat retention. We found it necessary to subcise the underlying fibrous connections in the fold. If the trabeculae between the fascia of the muscle and the skin were excised and the fat deposited into this pocket, the improvement was more substantial.

The lips also proved to be difficult areas of augmentation. The best augmentation was achieved with a multiple-layer procedure, starting on the mucosa side of the lip and working around the lips into the muscle and to the vermillion border. In this way, approximately 3–6 mL of tissue could be injected into each lip for each session. The lip may need other procedures such as lip advancement, permanent tattooing, or the use of more permanent implants such as Gortex®.[5]

In summary, the main difficulties with fat transfer continue to be partial reabsorption of the implants. We have developed guidelines to follow to provide more uniform, permanent augmentations (List 2). However, the technique is quite operator dependent and needs to be done on a frequent basis to achieve consistent results. Patients also need to plan two or three sessions to provide their level of satisfaction. If these guidelines are followed, it is possible to develop a satisfied patient base and to develop a tissue augmentation practice.

List 2. GUIDELINES OF FAT TRANSFER

1. Inform the patient that multiple sessions are planned, to wash with povidone-iodine, and to use no aspirin or other anti-inflammatories before the procedure.
2. Collect fat from concentrated donor site, such as the outer thigh. Tumesce the area and collect the fat with the least trauma. Use Mercedes tip and a vented syringe
3. Concentrate the fat through a sieve or centrifugation. Add bone powder for a firmer implant.
4. Place fat evenly throughout the tissue. Inject thin filaments of fat in a retrograde fashion into multiple tissue planes.
5. Support the donor and recipient sites with foam for several days to prevent hematoma formation. Use steroids and antibiotics to reduce the possibility of inflammation.
6. Wait 2–3 months between injection sessions. This gives adequate time for neovascularization.

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Comparative Study of Survival of Autologous Adipose Tissue Taken and Transplanted by Different Techniques

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In recent years, adipocytes obtained by suction-assisted lipectomy have been used for implantation by injection methods. This study is designed to assess the appearance of suctioned and excised adipose tissue and its survival after being injected or implanted into different tissues (0.5 cc into the rectus muscle and 0.5 cc into the dorsal ear skin) of New Zealand White rabbits. The results showed that significant numbers of adipocytes were ruptured after suction procedures. The intact cells represented approximately 10 percent of the fat cell population. Fat cells in aspirated and excised samples remained intact and did not differ histologically. After being injected into tissue, adipocytes appeared to survive better for a short term in a more vascularized bed (rectus muscle) than in a low vascular area (ear dermis). Long-term studies at 6- to 9-month intervals revealed transplanted adipose tissue, taken by suction or excision, being replaced with fibrosis, although cystic spaces and only a small number of surviving adipocytes were still present. Insulin did not show any protective effects on survival of the adipocytes during their transplantation.

Human adipose tissue transplantation was first attempted in 1893 by Neuber[1] and later by Lexer (1910),[2] who reported successful transplantation of fat to restore normal contour in the treatment of hemifacial atrophy and other skin deficiencies. Subsequently, adipose tissue transplantation was used widely as a surgical method to correct surface depression or to augment soft tissue during the early period of the twentieth century.[3-12] The details of histologic changes in the transplanted adipose tissue were described by Peer.[13] In his study, the adipose tissue grafts lost approximately 50 percent of their weight and volume 1 year or more following transplantation with normal-appearing adipose tissue. The cell survival theory is therefore based on the fact that some adipocytes failed to survive the trauma of grafting as well as the surgical trauma at the recipient site. Sidman[14] showed that the addition of insulin to the fat-cell culture medium in vitro may have inhibited lipolysis. Another proven effect of insulin on connective tissue was that adding insulin to a culture medium of fibroblasts induced the fibroblasts to take up free lipid droplets and become adipocytes.[15]

Recently, with the introduction of suction-assisted lipectomy to the plastic surgeon's armamentarium,[16] there has been revived interest in using suctioned adipose tissue for subcutaneous transplantation to correct contour deficiencies.[17-20] The purpose of this study was to evaluate adipocyte survival in two different types of tissue in rabbits after autologous transplantation (1) by injection technique after removing the adipose tissue by suction or aspiration and (2) by excision and implantation. Moreover, the beneficial effects of insulin, when added to the transplanted specimens, also were examined.

Materials and Methods

Twenty-eight New Zealand White male rabbits weighing 2.5 to 2.8 kg were used. Rabbits have an easily accessible fat deposit (fat pad) in the groin. The volume of each fat pad is about 3 to 4 cm³. Young rabbits have little or no adipose

From the Section of Plastic and Reconstructive Surgery, Department of Surgery, at the University of Michigan Medical School. Received for publication July 18, 1988; revised February 8, 1989.

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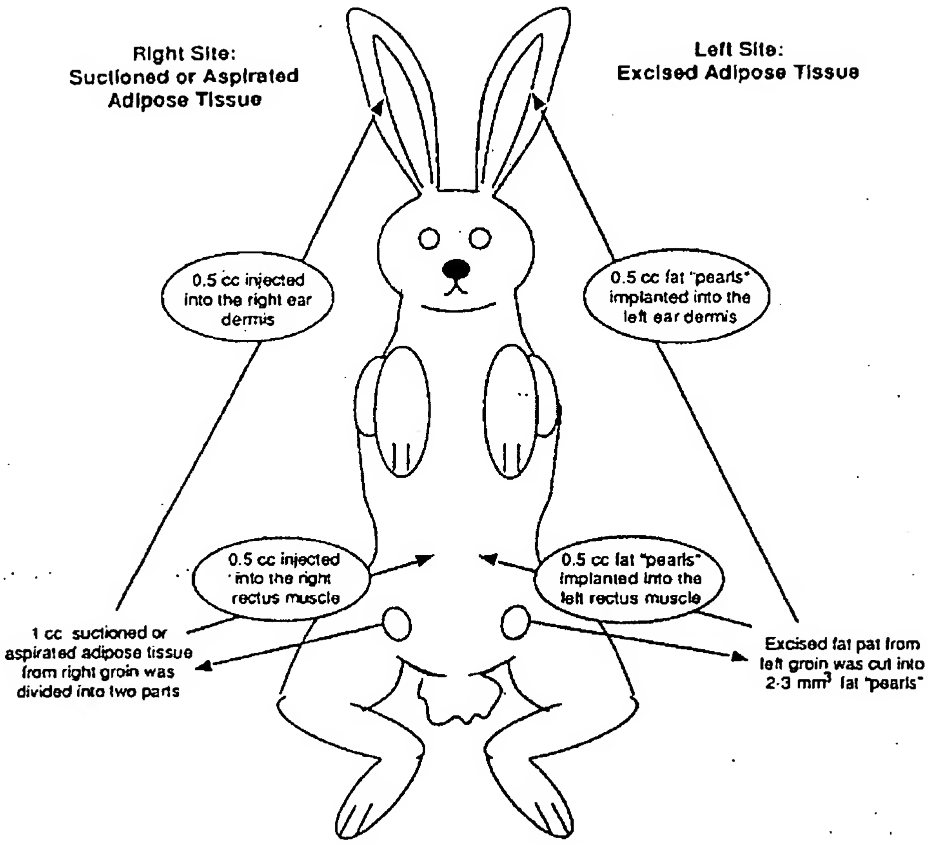


FIG. 1. Schema of autologous adipose tissue transplantation in the rabbit.

tissue in the ear subcutis and rectus muscle. Therefore, these areas were chosen as recipient sites for the adipose tissue.

Fat tissue was removed from the right groin fat pad by either standard fat suction using 1 atm negative pressure or by aspiration using a 14-gauge blunt needle and 10-cc syringe. Approximately 1 cc fragmented adipose tissue was suspended in physiologic saline at room temperature and immediately injected into the same animal using a 14-gauge blunt needle. Half (0.5 cc) the suctioned, as well as aspirated, adipose tissue was injected into the skin of the right dorsal ear. The other half (0.5 cc) was injected into the right rectus muscle, surgically prepared earlier and marked with 4-0 nylon black sutures for future orientation of specimen excision.

The left side of the animal was used as the

TABLE I
Transplantation of Autologous Adipose Tissue in 28 Rabbits

Excision Time after Transplantation	With Insulin				Without Insulin			
	No. of Animals	No. of Specimens			No. of Animals	No. of Specimens		
		Suctioned	Aspirated	Excised		Suctioned	Aspirated	Excised
2 Weeks	2	2	2	4	2	2	2	4
1 Month	2	2	2	4	2	2	2	4
2 Months	1	—	2	2	1	2	—	2
4 Months	4	4	4	8	3	4	2	6
6 Months	3	4	2	6	3	4	2	6
9 Months	2	2	2	4	3	4	2	6
TOTAL	14	14	14	28	14	18	10	28

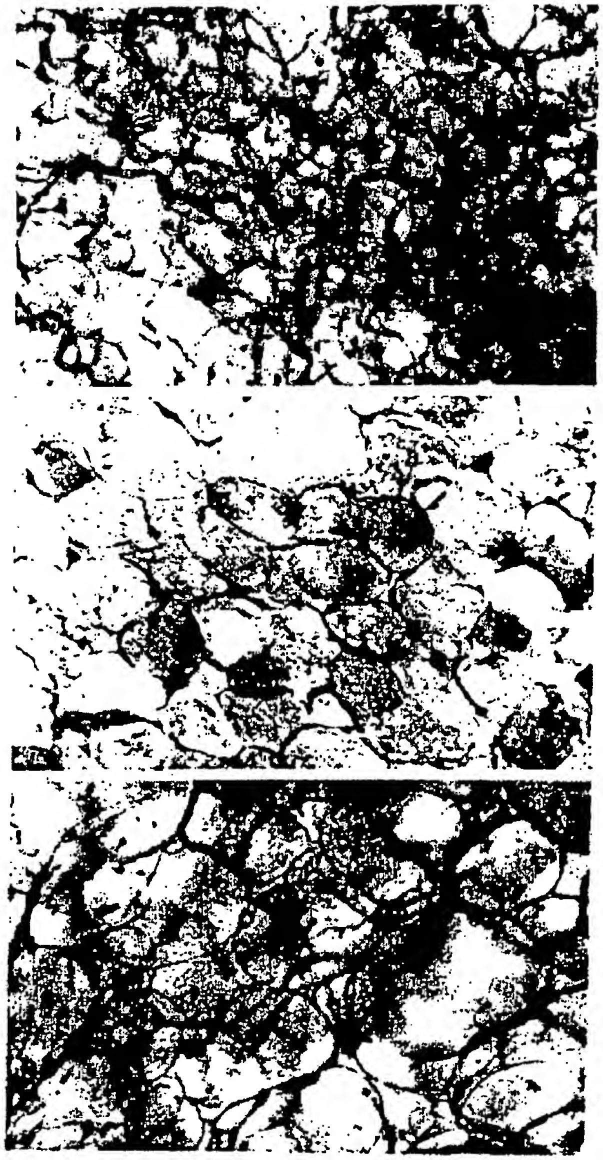


FIG. 2. (*Above*) Suctioned adipose tissue before transplantation. Note elongated, different in shape, and ruptured adipocytes as well as only a few normal adipocytes. Note also small stripes of connective tissue and fragmented blood vessels (H&E stain: × 125). (*Center*) Excised adipose tissue before transplantation with almost all unchanged adipocytes (H&E stain: × 125). (*Below*) Aspirated adipose tissue before transplantation. Note majority of unchanged adipocytes (H&E stain: × 125).

control side. From the left groin, a fat pad was excised and cut into 2- to 3-mm^3 pearls; then 0.5 cc was implanted into the skin of the left dorsal ear and 0.5 cc was implanted into a pocket made in the left rectus muscle, also marked earlier with sutures (Fig. 1).

In half of all suctioned, aspirated, and excised specimens, insulin (1 unit per 10 cc saline) was added to the suspension of fragmented or chopped adipose tissue.

For histopathologic examination, the adipose tissue, after suction, aspiration, and excision with and without insulin, was fixed in 10% buffered Formalin, embedded in Paraplast-Plus, and stained with hematoxylin and eosin and Wilder's silver stain for reticulin. Biopsy specimens from each injected and implanted area were taken after 2 weeks and 1, 2, 4, 6, and 9 months (Table I) and routinely prepared for light microscopic examination with hematoxylin and eosin staining and the von Kossa method for calcium.

Results

Histopathology of the Adipose Tissue before Transplantation

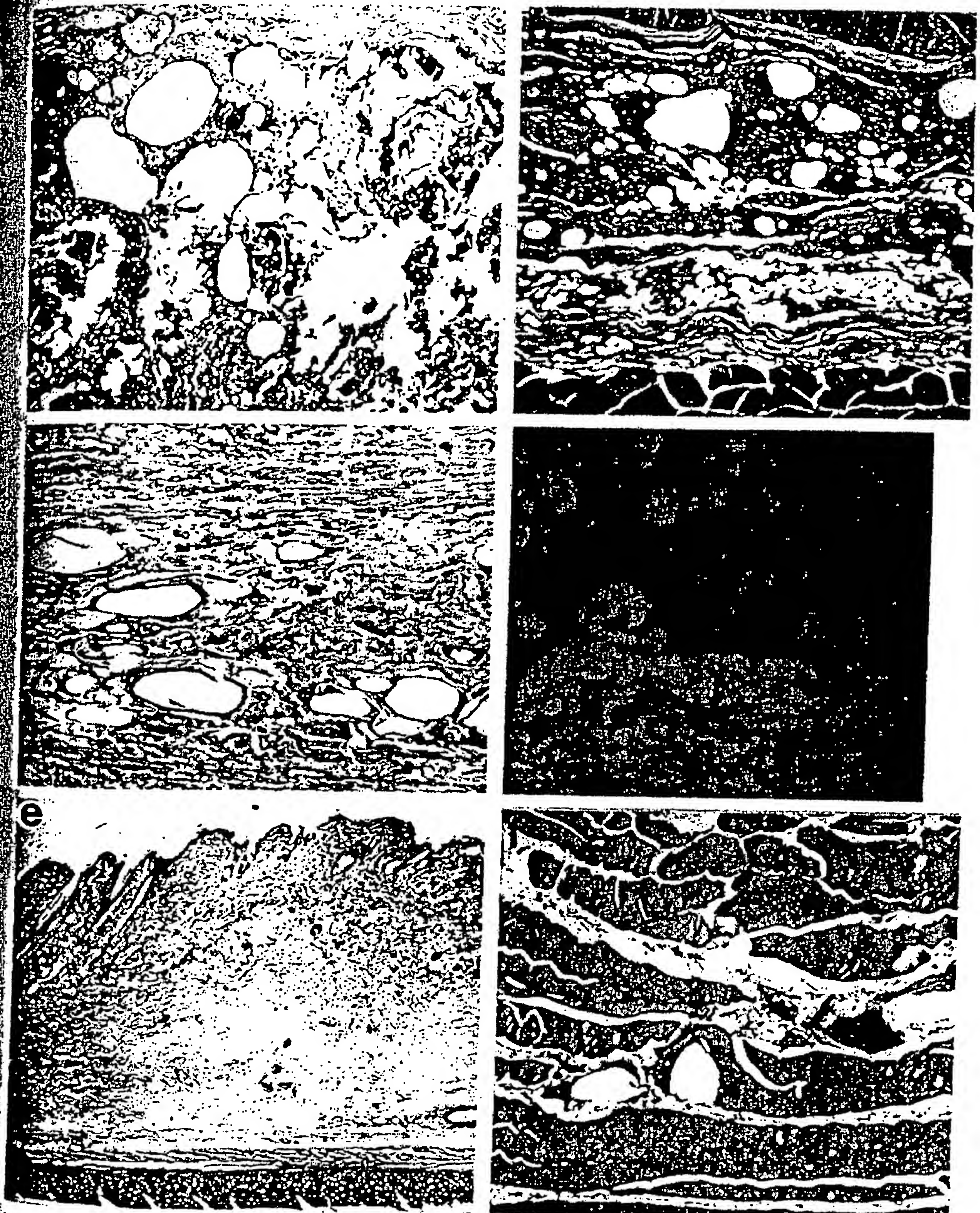
The suctioned adipose tissue examined with the light microscope was a mixture of fragments containing approximately 90 percent elongated, irregularly shaped, and ruptured adipocytes and only 10 percent unchanged, normal-appearing adipocytes (Fig. 2, *above*). The reticulin network in Wilder's staining was irregular and shattered.

In the aspirated and excised specimens, the unchanged adipocytes represented the majority, i.e., approximately 95 percent of the fat cell population (Fig. 2, *center* and *below*). In all specimens, fragmented stripes of small blood vessels and collagen fibers were found.

Histopathology of the Adipose Tissue after Transplantation

Areas of Injection of Suctioned Adipose Tissue. In all specimens taken from the ear and rectus muscle up to 2 months after injection of fragmented adipose tissue, there was focal necrosis of adipocytes, irregular size and shape of cystlike cavities,

FIG. 3. Suctioned adipose tissue after implantation. (*Above, left*) One month after injection of adipose tissue into the skin of the ear. Note necrosis, irregular cystlike spaces, macrophages, giant cells, lymphocytes, and only a few unchanged adipocytes (H&E stain; × 80). (*Above, right*) Two months after injection of adipose tissue into the rectus muscle. Note foreign-body reaction with giant cells and macrophages, cystic cavities, necrosis, and small groups of normal adipocytes, as well as inflammatory reaction and proliferation of fibroblasts (H&E stain; × 63). (*Center, left*) Injected adipose tissue into the ear skin 6 months later showing cystlike spaces and a few adipocytes between thick bundles of collagen fibers, macrophages, and giant cells (H&E stain; × 80). (*Center, right*) Six months after injection of adipose tissue into the rectus muscle. Note adipocytes in



very small number as well as cystic cavities surrounded by macrophages and fibrotic tissue (H&E stain; × 200). (*Below, left*) Nine months after injection of suctioned adipose tissue into the ear dermis. No surviving adipose tissue is present. Note scar tissue in the center (H&E stain; × 12). (*Below, right*) Suctioned adipose tissue 9 months after injection into the rectus muscle. No viable adipocytes are present, but cystic spaces and macrophages still are visible (H&E stain; × 50).

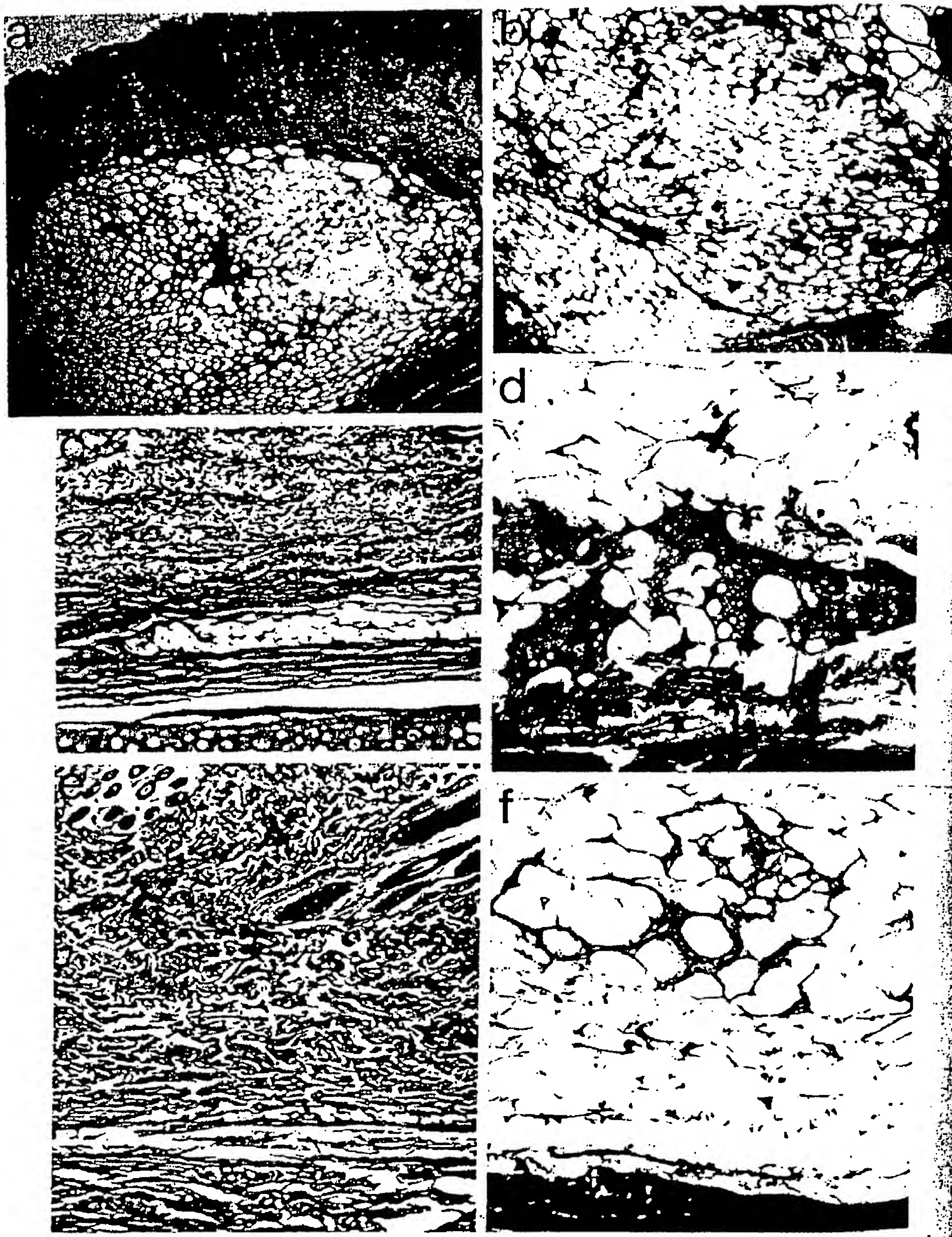


FIG. 4. Aspirated adipose tissue after transplantation. (*Above, left*) Adipose tissue inside the dermis 1 month after injection showing areas of necrosis, cystic spaces, foreign-body reaction, lymphocytic infiltration, and fibrosis (H&E stain; × 12). (*Above, right*) Adipose tissue inside the rectus muscle 1 month after injection. Note necrotic cells, cystlike cavities, macrophages

and only a few unchanged adipocytes. All were surrounded by inflammatory cells, macrophages filled with lipid droplets, giant cells, and in some part by fibroblasts. This foreign-body reaction and inflammation occurred more often in the dermis of the ear than in the rectus muscle (Fig. 3, *above, left* and *right*).

In specimens taken 4 and 6 months after injection of suctioned adipose tissue, only a few adipocytes were found. There was still the presence of foreign-body reaction and successive increased fibrosis around adipocytes and cystic spaces both in the dermis of the ear and in the rectus muscle (Fig. 3, *center, left* and *right*).

Nine months after injection of suctioned adipose tissue, no recognizable adipocytes could be found either in the ear skin or in the rectus muscle (Fig. 3, *below, left* and *right*).

Areas of Injection of Aspirated Adipose Tissue. In the early stages of transplantation, between 2 weeks and 2 months, a mixture of adipocytes, empty cystlike spaces, macrophages, giant cells, fibroblasts, and lymphocytes, as well as neovascularization in some areas of the specimens, was seen (Fig. 4, *above, left* and *right*).

In specimens taken from the ear skin and the rectus muscle 4 and 6 months after injection of aspirated adipose tissue, only a few adipocytes were found surrounded by fibrotic tissue. The macrophages, giant cells, and cystic spaces were still visible (Fig. 4, *center, left* and *right*).

In the specimens taken 9 months after injection of aspirated adipose tissue, almost no adipocytes were present in the dermis of the ear (Fig. 4, *below, left*). In the rectus muscle, small areas with adipocytes and cystlike cavities were surrounded by fibrotic tissue (Fig. 4, *below, right*).

Areas of Implantation of Excised Adipose Tissue. In the early phase, i.e., 2 weeks to 2 months, transplanted adipocytes as well as cystic spaces were visible between macrophages, giant cells, fibroblasts, inflammatory cells, and neovascularized areas. Necrosis, foreign-body reaction, and inflammatory infiltrations were observed more often in the dermis of the ear than in the rectus muscle (Fig. 5, *above, left* and *right*).

At longer intervals, i.e., 4 to 6 months, the implanted adipocytes were successively replaced by connective tissue; however, macrophages and foreign-body giant cells still were visible (Fig. 5, *center, left* and *right*).

In the specimens taken 9 months after implantation of excised adipose tissue, only a few adipocytes and cystic spaces were present (Fig. 5, *below, left* and *right*).

There were no histopathologic differences in the adipocytes transplanted with or without insulin in all examined specimens. Also, calcification was not observed in any specimen.

Discussion

The behavior of the adipose tissue after transplantation has been described by many authors.[21-25] Peer[25] observed that the host histiocytes that invade all adipose tissue grafts appeared to serve only as scavengers in removing fat from ruptured adipocytes. There were significant numbers of giant cells and cystic spaces filled with lipid. The surviving adipocytes were surrounded with fibroblasts and lymphocytes. Peer also stated that the survival of adipocytes in autologous grafting depends on early revascularization, which was observed at the fourth day after transplantation. The need for revascularization for adipose tissue graft survival was again emphasized by Smahel.[24]

Humans can grow fat in most areas of the body. The rabbit ear and rectus muscle were chosen because repeated anatomic and histologic examinations showed that there was no adipose tissue in these areas in young rabbits. This enabled us to follow the progress of the grafted fat without trying to differentiate between native and grafted fat cells. The rectus muscle and the ear could represent highly vascularized and poorly vascularized areas in the human body, respectively.

Our findings in transplanted adipose tissue were consistent with histologic changes in Peer's study with the presence of necrotic fragments of tissue and cystlike cavities formed by extruded lipid from broken adipocytes surrounded by

lymphocytes, beginning process of fibrosis, and a few new blood vessels (H&E stain; × 25). (*Center, left*) Six months after injection of adipose tissue into the ear dermis showing very narrow stripe of adipocytes between thickened bundles of collagen fibers (H&E stain; × 63). (*Center, right*) Adipose tissue 6 months after injection into the rectus muscle. Note a few normal-looking adipocytes as well as cystic spaces, macrophages filled with lipid droplets, and fibrosis (H&E stain; × 125). (*Below, left*) Nine months after injection of adipose tissue into the ear dermis. Note only a few adipocytes inside the fibrous tissue (H&E stain; × 50). (*Below, right*) Suctioned adipose tissue 9 months after injection into the rectus muscle still showing fibrotic process surrounding adipocytes and cystic spaces (H&E stain; × 125).

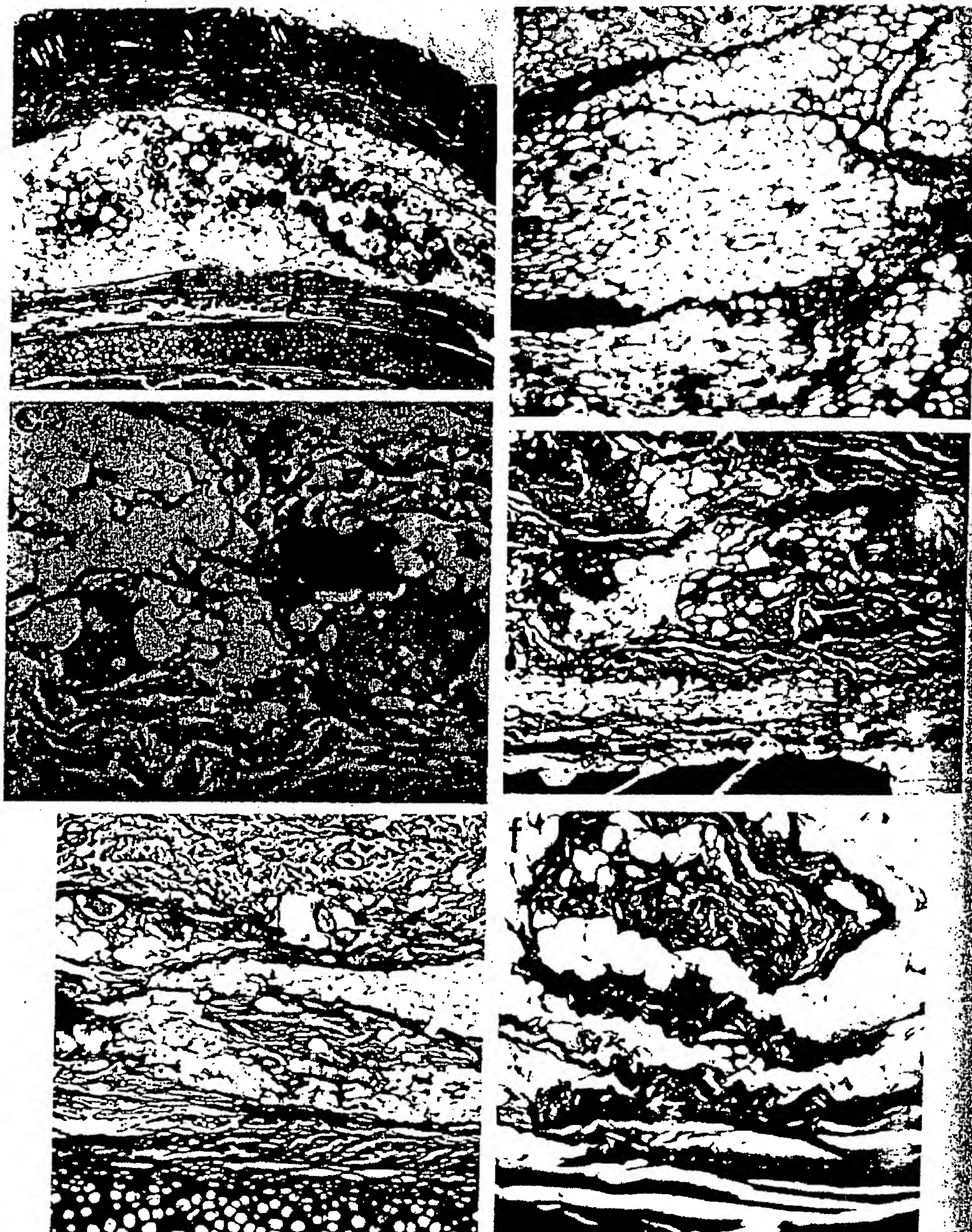


FIG. 5. Excised adipose tissue after transplantation. (*Above, left*) Adipose tissue 1 month after implantation into the skin on the ear with areas of necrosis, cystlike spaces, foreign-body reaction, and inflammation (H&E stain; × 15). (*Above, right*) One

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macrophages, giant cells, and inflammatory cells in early time frames. Revascularization observed in some border areas was connected with the formation of granulation tissue on the edge of the implant. Later, only proliferation of fibroblasts and increased fibrosis was observed. These changes, seen in both the injected and implanted areas, were most intense when suctioned adipocytes were injected. These findings were found in all samples taken from all rabbits studied; thus there was no histologic variation between these animals.

Our observations of suctioned adipose tissue before injection under the light microscope were quite different from those of Bircoll[19] and Courtiss,[26] who noted essentially normal intact or occasionally distorted adipocytes after the liposuction technique. We found unchanged adipocytes with intact cell membrane only in approximately 10 percent of the fat cell population in the suctioned samples. The remaining tissue was a mixture of abnormally shaped, ruptured adipocytes and fragmented small blood vessels, as well as loose connective tissue and lipid droplets located outside the cells. The ruptured adipocytes in the suctioned samples appeared to sustain mechanical damage. The question of the fragility of the rabbit fat cell as compared with its human counterpart remains unknown; however, damage to the adipocytes was probably due to high negative suction pressure and low melting point in the suction tubing. Insulin did not show any protective effect on the adipocyte cell membrane. So, in the first stage of implantation, intensive foreign-body reaction and inflammation take place. This is "histologic cleaning" of necrotic fragments of the tissues and removal of the free lipid droplets from the ruptured adipocytes by macrophages. Later, the long-term reparation process begins with proliferation of fibroblasts and production of fibrosis.

In the skin of the ears of both experimental and control sides, the grafted adipose tissue was largely phagocytized by macrophages and surrounded by collagen fibers, and finally, the whole graft was replaced by fibrous connective tissue. The adipocytes, therefore, did not appear to survive in the dermis of the ear, which has low vascularity.

The aspirated and excised adipose tissue in transplanted areas showed a reaction similar to that of suctioned and injected fragments of adipose tissue. There were both a foreign-body reaction and inflammation in the areas of transplantation. At long intervals, from 6 to 9 months, almost all grafted adipocytes in the ear dermis were replaced by fibrosis. In the highly vascularized rectus muscle, however, a small number of adipocytes was still found scattered in the fibrotic tissue, thus implying the importance of vascularization of the recipient site.

Peer[23] and Smahel[24] had indicated that there was up to 50 to 60 percent loss of weight and reduction in volume of the adipose tissue 3 months to 1 year after transplantation. Our study showed that the majority of grafted adipose tissue 9 months after transplantation was replaced by fibrosis. Because the viable adipocytes were minimal at that time of observation, we cannot conclude that adipose tissue can be transplanted to correct soft-tissue deficiency. It is possible, however, that cystic cavities and fibrous connective tissue are the main components that maintain the bulk and tissue volume after long-term adipose tissue transplantation.

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month after implantation of adipose tissue into the rectus muscle. Note lobules of adipose tissue with necrotic areas, inflammation, and beginning process of fibrosis (H&E stain; × 31). (*Center, left*) Six months after implantation of adipose tissue into the skin. Note small amount of adipocytes, cystic cavities, giant cells, and fibrosis (H&E stain; × 157). (*Center, right*) Six months after implantation of adipose tissue into the rectus muscle. Note very few viable adipocytes, cystic spaces, foreign-body reaction, and fibrosis (H&E stain; × 50). (*Below, left*) Adipose tissue 9 months after implantation into the skin of the ear. Note very small amount of viable adipose tissue, cystic spaces, and replacement of adipocytes by fibrosis (H&E stain; × 50). (*Below, right*) Nine months after implantation of adipose tissue into the rectus muscle showing similar changes as in the dermis. Note significant fibrosis and very small number of adipocytes (H&E stain; × 50).

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